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PROVISIONAL APPLICATION COVER SHEET

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	Docket Number	3665-84	Type a plus sign (+) inside this box→	+

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TITLE OF THE INVENTION (280 characters)

COMPOSITION AND METHOD FOR THE TREATMENT OF CARCINOMA

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification	Number of Pages	56	☐ Applicant claims "small entity" status. ☐ "Small entity" statement attached.		
☐ Drawing(s)	Number of Sheets		☒ Other (specify)	32 Numbered Claims 1 Page Abstract	

METHOD OF PAYMENT *(check one)*

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The invention was made by an agency of the United States Government or under a contact with an agency of the United States Government.

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Respectfully submitted,
SIGNATURE B J Sadoff

DATE **February 10, 2004**

REGISTRATION NO. *(if appropriate)* 36,663

TYPED or PRINTED NAME B. J. Sadoff

☐ Additional inventors are being named on separately numbered sheets attached hereto.

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COMPOSITION AND METHOD FOR THE TREATMENT OF CARCINOMA

FIELD OF THE INVENTION

The present invention relates to compositions and methods useful for treating a carcinoma or viral infection in mammals, including humans. The methods and compositions typically comprise a Mycobacterium antigen, and a γδT cell activator, such that the composition is effective for treating a carcinoma or viral infection. In a preferred aspect of the invention, the Mycobacterium antigen is an attenuated strain of Mycobacterium bovis (Bacillus Calmette-Guérin (BCG)). Methods of making the composition and methods of using it also are disclosed.

BACKGROUND OF THE INVENTION

Carcinomas account for about 85% of all cancers. A significant portion of these carcinomas are carcinoma in situ, or superficial cancers, such as superficial bladder cancer and diseases caused by human papilloma virus (HPV) infection.

Bladder cancer

Carcinoma of the bladder accounts for about 2 % of all solid tumors in the United States with more than 50,000 new cases being diagnosed each year. The peak prevalence of bladder cancer is in individuals 60-70 years old and several etiologic factors have been implicated including smoking and exposure to industrial chemicals. Bladder cancer is the fifth most common neoplasm and the twelfth leading cause of cancer death.

Pathologically, carcinoma of the bladder is categorized by grade (usually I-IV) and by depth of malignancy (either superficial, invasive, or metastatic bladder cancer). Superficial bladder cancer, which is confined to the bladder epithelium, usually presents as papillary tumors (stages ta or T1) or carcinoma-in-situ (CIS). Diagnosis of bladder cancer is by cytoscopy and biopsy. At the time of diagnosis, about 70 % of patients have only superficial disease, 25 % have locally invasive disease, and 5 % already have distant metastasis.

Superficial bladder cancer is treated with transurethal resection and/or fulguration. Cytoscopy is usually reserved for those tumors which cannot be resected transurethrally. After transurethal resection, 50 % of patients remain disease free; however the other half will experience multiple recurrences with about 10 % developing invasive or metastatic disease within 3-4 years. Superficial recurrences are treated with transurethal resection, often followed by intravesical

chemotherapy to prevent or delay any additional recurrence. Patients who are considered at high risk for recurrence after the initial transurethal resection or those with concurrent CIS are frequently given intravesical adjunct therapy as prophylaxis against recurrence.

High risk patients are candidates for intravesical therapy with bacillus Calmette-Guerin (BCG), mitomycin, doxorubicin or thiotepa. These agents are typically instilled into the bladder through a urethral catheter for two hours weekly for six to eight weeks. Occasionally, long-term maintenance treatment regimens are employed.

Clinical studies may have various endpoints such as tumor recurrence, tumor progression or patient survival. In clinical trials comparing transurethral resection plus and an intravesical agent versus transurethral resection alone, a significant reduction in tumor recurrences was noted in 4 of 5 BCG studies, 2 of 5 mitomycin studies, 2 of 4 doxorubicin studies, and 6 of 10 thiotepa studies; and a significant reduction in tumor progression was documented in 3 of 3 BCG studies, 0 of 2 mitomycin studies, 0 of 2 doxorubicin studies, and 0 of 3 thiotepa studies. Among these agents, BCG is the only one shown to result in a survival advantage over transurethral resection alone. (Herr et al, J Clin Oncol 1995, 13, 1404-8; Sarosdy & Lamm, J Urol 1989, 142, 719-22; Catalona et al, J Urol 1987, 137, 220-4; De Jager et al, Urology 1991, 38, 507-13; Herr et al, J Urol 1992, 147, 1020-3).

The mechanism of action of BCG in treatment of bladder cancer is unknown. However, the available evidence suggests that intravesical BCG is a form of immunotherapy. Intravesical BCG appears to induce tumor regression through a number of specific and non-specific actions. It promotes a local inflammatory reaction with histiocytic and leukocytic infiltration in the urinary bladder that is apparently associated with an elimination or reduction of superficial cancerous lesions.

Although BCG treatment of bladder cancer is efficient, there remain approximately 40% of patients for whom such treatment does not result in disappearance of the cancer. There is therefore a need in the art for more efficacious therapy of bladder cancer.

HPV infection

Human papilloma virus (HPV) infections of the urogenital tract represent the most often sexually transmitted viral disease in humans. HPV is a double stranded DNA virus and with the recent developed molecular biological techniques, more than 55 different HPV types have been recognized. HPV is associated with a wide spectrum of clinical states including condylomata

acuminata, latent and subclinical infection, and Bowen's disease. Subclinical infections gain more importance as they are believed to cause intraepithelial neoplasia, based on the frequent detection of HPV DNA in invasive carcinomas, especially in urogenital region. A significant risk for the development of an invasive cancer is ascribed to the infections by HPV types 1 6, 1 8 and 33.

The most prevalent HPV types causing condylomata acuminata are type 6 and 11. Condylomata acuminata are visible, multifocal, multicentric and multiform lesions. Predilection sites are penis, scrotum, perineum, urethra, perianal regions, intertriginous zones, and oral mucosa. In uncircumcised men the frenulurn, the coronary sulcus and the inner aspect of the foreskin are most often afflicted, whereas in circumcised patients the shaft of the penis is involved. Genital warts are of great psychological and cosmetic relevance representing a major hindrance to sexual performance.

Treatment options include surgical methods like excision, electrocautery, cryosurgery or laser vaporization. It has been shown in molecular hybridization studies that HPV DNA sequences exist in adjacent normal tissue after carbon dioxide laser removal of genital warts. These findings and the well known high recurrence rates after initial treatment demonstrate the need for adjuvant therapy to eradicate invisible disease.

Therapeutic results with local application of cytotoxic agents, for example, 5-fluorouracil and podophyllin/podophyllotoxin. More recently, it been suggested that treatment with a mycobacterial antigen such as attenuated BCG composition may be efficacious against HPV-related disease (PCT patent publication no. WO9955347, the disclosure of which is incorporated by reference). Despite the availability of therapeutic agents, treatment of HPV related disease to date remains unsatisfactory in its efficacy and side effects.

SUMMARY

The present invention now discloses particular compositions and methods that can be used to efficiently treat a carcinoma, particularly an epithelial cell cancer, and preferably a bladder cancer, in a subject. The invention also provides compositions and methods for the treatment of a viral infection, preferably an HPV infection, and conditions associated therewith.

In one aspect, the inventors have provided a method for treatment which involves administering locally to a site of disease a composition capable of recruiting or preferably regulating γδ T cell

activity. Any suitable γδ T cell activating compound can be used in such a composition for local administration, including a range of γδ T cell activating compounds described herein. The most well known (currently approved for human therapy) examples for local administration include the mycobacterial antigens. In conjunction with this local administration, a γδ T cell activating compound that stimulates the proliferation and/or biological activity of γδ T cells is administered to the patient by a non-local route, preferably systemically. The γδ T cell activating compound administered systemically can be the same compound as the γδ T cell activating compound administered locally, or can be a different compound. However, when a mycobacterial strain is used as the locally administered compound, it will be preferably to use a different γδ T cell activating compound for systemic administration. A wide variety of preferred γδ T cell activating compounds for systemic administration, particularly synthetic and selective γδ T cell activating compounds are provided herein. This combination therapy thereby amplifies the γδ T cell-mediated effects of the composition that is administered locally.

The invention thus provides a method for the treatment of a disease comprising:

(a) administering to said subject a first γδ T cell activator compound in a quantity sufficient to stimulate γδ T cell activity; and

(b) administering to a subject locally at a site of disease, a second γδ T cell activator, said second γδ T cell activator being administered and in a quantity effective to treat said disease when used in combination therapy with said first γδ T cell activator. Said first and second γδ T cell activators may comprise the same compound or composition or may comprise different compounds or compositions. Preferably the first γδ T cell activator is administered systemically, preferably by intravenous injection. In a preferred aspect, the second γδ T cell activator comprises a mycobacterial antigen. Said disease is preferably a carcinoma or a viral infection; preferred examples include respectively a bladder cancer or an HPV infection.

In a preferred embodiment, the invention provides a combination therapy in which a mycobacterial antigen is delivered locally to a site of disease and a γδ T cell activator is administered by a second, non-local route of administration. The invention provides a method for the treatment of a disease comprising:

(c) administering to said subject a γδ T cell activator compound in a quantity sufficient to stimulate γδ T cell activity; and

(d) administering to a subject a mycobacterial antigen, said antigen being administered locally to a site of disease and in a quantity effective to treat said disease when used in combination therapy with said γδ T cell activator.

Preferably, the γδ T cell activator is not administered locally to a site of disease; for example a γδ T cell activator may be administered systemically, preferably by intravenous route. Said disease is preferably a carcinoma or a viral infection; preferred examples include respectively a bladder cancer or an HPV infection.

The also invention discloses a method for treating bladder cancer or HPV infection in a patient comprising administering to a patient in need thereof an amount of a Mycobacterium antigen and a γδ T cell activator effective to treat said disease. The invention also discloses a pharmaceutical composition comprising a Mycobacterium antigen and a γδ T cell activator, preferably at an effective dose to treat a carcinoma such as bladder cancer, urinary cancer or a viral infection such as an HPV infection.

The invention further discloses the use of a Mycobacterium antigen and a γδ T cell activator for the manufacture of a pharmaceutical composition for the treatment of bladder cancer.

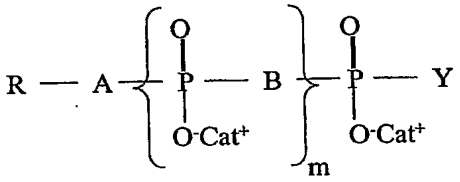
The invention finally discloses a kit for the treatment of bladder cancer comprising a Mycobacterium antigen and a γδT cell activator.

Preferably, said Mycobacterium antigen is an antigen of Mycobacterium bovis. Alternatively, said Mycobacterium antigen is an antigen of Mycobacterium phlei. More preferably, said Mycobacterium antigen is an attenuated strain thereof. Still more preferably, said Mycobacterium antigen is an attenuated strain of Mycobacterium bovis (BCG). In a particular embodiment, said Mycobacterium antigen is mycobacterial cell wall, preferably complexed to Mycobacterium DNA.

Preferably a γδ T cell activator is a compound capable of regulating the activity of a γδ T cell in a population of γδ T cell clones in culture. The γδ T cell activator is preferably capable of regulating the activity of a γδ T cell population of γδ T cell clones at millimolar concentration, preferably when the γδ T cell activator is present in culture at a concentration of less than 100 mM. Optionally a γδ T cell activator is capable of regulating the activity of a γδ T cell in a population of γδ T cell clones at millimolar concentration, preferably when the γδ T cell activator is present in culture at a concentration of less than 10 mM, or more preferably less than

1 mM. Regulating the activity of a γδ T cell can be assessed by any suitable means, preferably by assessing cytokine secretion, most preferably TNF-α secretion as described herein. Methods for obtaining a population of pure γδ T cell clones is described in Davodeau et al, (1993) J. Immunology 151(3): 1214-1223 and Moreau et al, (1986) J. Clin. Invest. 78:874, the disclosures of which are incorporated herein by reference. Preferably the activator is capable of causing at least a 20%, 50% or greater increase in the number of γδ T cells in culture, or more preferably at least a 2-fold increase in the number of γδ T cells in culture.

Preferably, said γδT cell activator is a composition comprising a compound of formula (I) :



Formula (I)

wherein Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including proton);

m is an integer from 1 to 3;

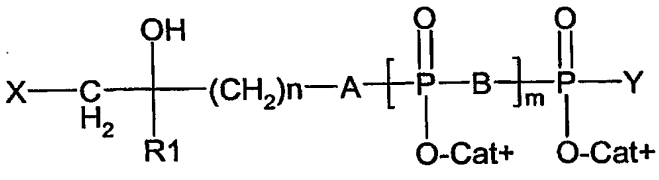
B is O, NH, or any group capable to be hydrolyzed;

Y = O^-Cat^+, a C_1-C_3 alkyl group, a group -A-R, or a radical selected from the group consisting of a nucleoside, an oligonucleotide, a nucleic acid, an amino acid, a peptide, a protein, a monosaccharide, an oligosaccharide, a polysaccharide, a fatty acid, a simple lipid, a complex lipid, a folic acid, a tetrahydrofolic acid, a phosphoric acid, an inositol, a vitamin, a co-enzyme, a flavonoid, an aldehyde, an epoxyde and a halohydrin;

A is O, NH, CHF, CF_2 or CH_2; and,

R is a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C_1-C_{50} hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkylenyl, or an alkynyl, preferably an alkyl or an alkylene, which can be substituted by one or several substituents selected from the group consisting of : an alkyl, an alkylenyl, an alkynyl, an epoxyalkyl, an aryl, an heterocycle, an alkoxy, an acyl, an alcohol, a carboxylic group (-COOH), an ester, an amine, an amino group ($-NH_2$), an amide ($-CONH_2$), an imine, a nitrile, an hydroxyl (-OH), a aldehyde group (-CHO), an halogen, an halogenoalkyl, a thiol (-SH), a thioalkyl, a sulfone, a sulfoxide, and a combination thereof.

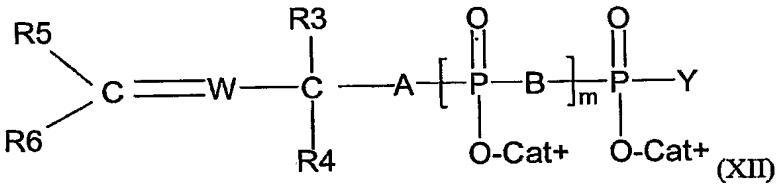
In a more preferred embodiment, said γδ T cell activator is a composition comprising a compound of formula (II):



(II)

in which X is an halogen (preferably selected from I, Br and Cl), B is O or NH, m is an integer from 1 to 3, R1 is a methyl or ethyl group, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), and n is an integer from 2 to 20, A is O, NH, CHF, CF_2 or CH_2, and Y is O^-Cat+, or a nucleoside. Still more preferably, said γδ T cell activator is selected from the group consisting of BrHPP, CBrHPP and epoxPP. Optionally, said γδ T cell activator is BrHPP. Alternatively, said γδ T cell activator is CBrHPP. Otherwise, said γδ T cell activator is epoxPP.

In an other more preferred embodiment, said γδ T cell activator is a composition comprising a compound of formula (XII):



(XII)

in which R_3, R_4, and R_5 , identical or different, are a hydrogen or $(C_1\text{-}C_3)$alkyl group, W is –CH- or –N-, R_6 is an $(C_2\text{-}C_3)$acyl, an aldehyde, an $(C_1\text{-}C_3)$alcohol, or an $(C_2\text{-}C_3)$ester, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), B is O or NH, m is an integer from 1 to 3, A is O, NH, CHF, CF_2 or CH_2, and Y is O^-Cat+, or a nucleoside. Still more preferably, said γδ T cell activator is selected from the group consisting of HDMAPP and CHDMAPP. In a first most preferred embodiment, said γδ T cell activator is HDMAPP. In a second one, said γδ T cell activator is CHDMAPP.

In a preferred embodiment, said Mycobacterium antigen and γδT cell activator are administered within about 48 hours, or about 24 hours of one another. Optionally said Mycobacterium antigen and γδT cell activator are administered simultaneously. Suitable treatment regimens may specify that the γδT cell activator can be administered before or after said Mycobacterium antigen. Said Mycobacterium antigen and γδT cell activator can be administered by the same routes. Alternatively, said Mycobacterium antigen and γδT cell activator can be administered by different routes. In a most preferred embodiment, the Mycobacterial antigen is administered locally to a site of disease and a γδT cell activator is administered by a non-local route,

preferably by systemic administration. Preferably, for the treatment of bladder carcinoma, said Mycobacterium antigen is administered by intravesicularly into the bladder. Preferably, said Mycobacterium antigen is administered after a transurethal resection, still more preferably 1 or 2 weeks after.

In a preferred embodiment, said bladder cancer is a stage 0 bladder cancer. More preferably, said stage 0 bladder cancer is a non-invasive papillomary carcinoma (TaT1) or a carcinoma in situ (CIS).

DETAILED DESCRIPTION

DEFINITIONS

Where "comprising" is used, this can preferably be replaced by "consisting essentially of", more preferably by "consisting of".

Where hereinbefore and hereinafter numerical terms are used, they are meant to include the numbers representing the upper and lower limits. For example, "between 1 and 3" stands for a range "from and including 1 up to and including 3", and "in the range from 1 to 3" would stand for "from and including 1 up to and including 3". The same is true where instead of numbers (e.g. 3) words denoting numbers are used (e.g. "three").

"Weekly" stands for "about once a week" (meaning that more than one treatment is made with an interval of about one week between treatments), the about here preferably meaning +/-1 day (that is, translating into "every 6 to 8 days"); most preferably, "weekly" stands for "once every 7 days".

"3-weekly" or "three-weekly" stands for "about once every three weeks" (meaning that more than one treatment is made with an interval of about three weeks between treatments), the about here preferably meaning +/-3 days (that is, translating into every 18 to 24 days); most preferably, "weekly" stands for "once every 21 days" (=every third week).

Whenever within this whole specification "treatment of a cancer" or the like is mentioned with reference to the compositions according the present invention, essentially a Mycobacterium antigen and a γδT cell activator, there is meant:

a) a method of treatment (=for treating) of a cancer, said method comprising the step of administering (for at least one treatment) a Mycobacterium antigen and a γδT cell activator, (preferably each in a pharmaceutically acceptable carrier material) to a mammal, especially a human, in need of such treatment, in a dose that allows for the treatment of said cancer (=a therapeutically effective amount), preferably in a dose (amount) as specified to be preferred hereinabove and hereinbelow;

b) the use of a Mycobacterium antigen and a γδT cell activator for the treatment of a cancer; or a Mycobacterium antigen and a γδT cell activator, for use in said treatment (especially in a human);

c) the use of a Mycobacterium antigen and a γδT cell activator, for the manufacture of a pharmaceutical preparation for the treatment of a cancer; and/or

d) a pharmaceutical preparation comprising a dose of a Mycobacterium antigen and a γδT cell activator that is appropriate for the treatment of a cancer; or any combination of a), b), c) and d), in accordance with the subject matter allowable for patenting in a country where this application is filed;

e) a method of using a Mycobacterium antigen and a γδT cell activator for the manufacture of a pharmaceutical preparation for the treatment of a cancer, comprising admixing said Mycobacterium antigen and said γδT cell activator(s) with a pharmaceutically acceptable carrier. It will be appreciated that references for example to treatment of a tumor, carcinoma, bladder cancer, urinary cancer, viral infection or HPV infection can be substituted in the above definition in the same way as the term cancer, and such twill be understood according to the same above definition as exemplified for cancer.

Within the context of the present invention, the expressions "stimulating the activity of γδ T cells" and "regulating the activity of γδ T cells" designate causing or favoring an increase in the number and/or biological activity of such cells in a subject. Stimulating and regulating thus each include without limitation modulating (e.g., stimulating) expansion of such cells in a subject and/or, for instance, triggering of cytokine secretion (e.g., TNFα or IFNγ). γδ T cells normally represent between about 1-10% of total circulating lymphocytes in a healthy adult human subject. The present invention can be used to significantly increase the γδ T cells population in a subject, particularly to reach at least 30% of total circulating lymphocytes, typically 40%, more preferably at least 50% or 60%, or from 50%-90%. Regulating also includes, in addition or in the alternative, modulating the biological activity of γδ T cells in a subject, particularly their cytolytic activity or their cytokine-secretion activity. The invention defines novel conditions and strategies for increasing the biological activity of γδ T cells towards target cells.

The present invention concerns a method for inhibiting the growth of cancer cells in the urinary bladder of a mammal having a bladder cancer comprising administering a Mycobacterium antigen and a γδT cell activator.

The present invention also concerns a method of preventing, or treating a carcinoma or viral infection, preferably a urinary or bladder cancer or an HPV infection in a mammal comprising administering a Mycobacterium antigen, and a γδT cell activator.

The present invention further concerns a pharmaceutical composition comprising a Mycobacterium antigen, and a γδT cell activator and the use thereof for treatment or prevention of carcinoma or viral infection, preferably a urinary or bladder cancer or an HPV infection. Optionally, said composition is prepared for separate administration of said Mycobacterium antigen and said γδT cell activator. Optionally, said composition further comprises a cytokine. Optionally, said composition also comprises an agent active against carcinoma or viral infection, preferably a urinary or bladder cancer or an HPV infection. Such agent includes, but are not limited to, drugs, immunostimulants, antigens, antibodies, vaccines, radiation and chemotherapeutic, genetic, biologically engineered and chemically synthesized agents, and agents that target cell death molecules for activation or inactivation and that inhibit proliferation of and induce apoptosis in responsive cells.

The present invention concerns a pharmaceutical composition comprising a Mycobacterium antigen, and a γδT cell activator for use as a medicament. More particularly, the invention concerns the use of a Mycobacterium antigen and a γδT cell activator for the manufacture of a medicament for the treatment of carcinoma or viral infection, preferably a urinary or bladder cancer or an HPV infection.

The invention also contemplates the methods and the compositions comprising several Mycobacterium antigens and/or several γδ T cell activators.

In a first embodiment, said Mycobacterium antigen and said γδT cell activator are administered simultaneously to said mammal. More particularly, a pharmaceutical composition comprising said Mycobacterium antigen and said γδT cell activator is administered to said mammal. In preferred aspects, said Mycobacterium antigen and said γδT cell activator can be administered by separately and are administered by different routes of administration, for example the

mycobacterial antigen is administered locally at a disease site and the γδT cell activator is administered systemically, preferably by intravenous (iv) route. Said Mycobacterium antigen can be administered to said mammal before or after said γδT cell activator.

In further preferred aspects, the methods may comprise further administering a cytokine. Said cytokine is capable of increasing the expansion of a γδ T cell population treated with a γδ T cell activator compound. A preferred cytokine is an interleukin-2 polypeptide (e.g., Research Diagnostics, NJ, #RDI-202). For example, cytokines for use in accordance with the invention and regimens for their administration are described is PCT patent publication no WO 01/56387, the disclosure of which is incorporated herein by reference.

The present invention more particularly concerns a freeze-dried (lyophilized) pharmaceutical composition comprising a Mycobacterium antigen, and a γδT cell activator. Preferably, the pharmaceutical composition according to the present invention is administered as an aqueous suspension. For administration in an aqueous carrier, the pharmaceutical composition according to the present invention is suspended in a pharmaceutically acceptable buffer including, but not limited to, saline and phosphate buffered saline (PBS) and is either asceptically processed or terminally sterilized. For example, freeze-dried (lyophilized) pharmaceutical composition according to the present invention may be stored in sealed ampoules or vials requiring only the addition of a carrier, for example sterile water, immediately prior to use.

Therefore, the present invention also concerns a kit comprising at least one container and a pharmaceutical composition according to the present invention. Preferably, containers are sealed ampoules or vials. Optionally, the kit can comprise a syringe. The kit can comprise a container comprising both Mycobacterium antigen, and γδT cell activator. The kit can also comprise a container comprising the Mycobacterium antigen and an other one comprising the γδT cell activator. Preferably, the pharmaceutical composition is freeze-dried (lyophilized).

Preferably, the pharmaceutical composition according to the present invention, in combination with a pharmaceutically acceptable carrier, is administered to a mammal locally to a site of disease in a dosage effective to treat the carcinoma. For example, in bladder cancer, local administration refers to administration into the bladder

Routes of administration include, but are not limited to, oral, topical, subcutaneous, percutaneous, intramuscular, intraperitoneal, intravenous, intradermal, intrathecal, intralesional, intratumoral, intrabladder, intra-vaginal, intraocular, intrarectal, intrapulmonary, intraspinal,

transdermal, subdermal, placement within cavities of the body, nasal inhalation, pulmonary inhalation, impression into skin and electrocorporation.

Any suitable method for administering the mycobacterial antigen can be used, depending on the disease. For example, for bladder cancer, preferred methods are as follows. The Mycobacterium antigen is administered by instillation into the urinary bladder by, but not limited to, a urinary tract catheter. Other methods for instilling the pharmaceutical composition according to the present invention into the urinary bladder are known to those skilled in the art. The γδT cell activator is provided by systemic administration, preferably by intravenous infusion. The bladder cancer is preferably a stage 0 bladder cancer. More preferably, the bladder cancer is a non-invasive papillomary carcinoma (TaT1) or a carcinoma in situ (CIS). More particularly, the methods of treatment and the pharmaceutical compositions according to the present invention are well adapted for the primary treatment of CIS of the bladder (after transurethial resection) either with or without associated papillary tumors, the secondary treatment of CIS of the bladder in patients treated with other intravesical agents who have relapsed or failed to respond, and the primary or secondary treatment of CIS in patients who have contraindications to radical surgery. Furthermore, these methods and compositions are also well adapted for the adjuvant treatment following transurethal resection of stage Ta or T1 papillary tumors of the bladder, which are at high risk of recurrence. In a preferred aspect of the present invention, the pharmaceutical compositions according to the present invention are administered to a subject having a bladder cancer after a step of transurethial resection. In a preferred embodiment, the treatment is administered 7-15 days after the transurethial resection.

Mycobacterium antigens

The Mycobacterial antigen for use according to the invention can be for example, and without to be limited thereto, live, killed or attenuated mycobacterium compositions, mycobacterial culture supernatants, mycobacterial cell extracts, cell wall fractions or cell wall elements, preferably purified cell wall elements, DNA fractions or purified DNA molecules, or mycobacterial peptide or non-peptidic (for example non-peptidic phosphorylated antigens) antigens, in the form of fractions enriched in said antigen or purified antigen. In addition to using attenuated or killed mycobacterial strains, it is also possible to use any of a variety of non-pathogenic or non-human infecting strains of Mycobacterium, such as for example M. phlei, M. piscium or M. smegmatis in naturally existing forms. Preferred mycobacterial antigens are compositions capable of regulating, preferably stimulating γδT cell activity.

In a preferred embodiment, said Mycobacterium antigen is an antigen of any one of Mycobacterium strains, more particularly a Mycobacterium strain selected from the group consisting of Mycobacterium avium, Mycobacterium bovis, Mycobacterium phlei, Mycobacterium tuberculosis, Mycobacterium paratuberculosis, Mycobacterium heamophilum, Mycobacterium leprae, Mycobacterium chelonae, Mycobacterium fortuitum, Mycobacterium kansasii, Mycobacterium marinum, Mycobacterium scrofulaceum, Mycobacterium smegmatis, Mycobacterium ulcerans, and Mycobacterium xenopi. More preferably, said Mycobacterium antigen is an antigen of a Mycobacterium strain selected from the group consisting of Mycobacterium bovis, Mycobacterium phlei, Mycobacterium tuberculosis, and Mycobacterium paratuberculosis. In a most preferred embodiment, said Mycobacterium antigen is an antigen of Mycobacterium bovis. In an other preferred embodiment, said Mycobacterium antigen is an antigen of Mycobacterium phlei, Mycobacterium vaccae or Mycobacterium piscium.

Preferably, said antigen of Mycobacterium is an attenuated strain thereof. Methods to prepare an attenuated strain are well-known by the man skilled in the art. For example, one method is disclosed in US 6,403,100. The Mycobacterium antigen can be for example any attenuated strain of Mycobacterium bovis (Bacillus Calmette-Guérin - BCG). Mycobacterium bovis strain can be a BCG strain of ATCC number selected from the group consisting of: 19015; 19274; 27289; 27290; 27291; 35731; 35732; 35733; 35734; 35735; 35736; 35737; 35738; 35739; 35740; 35741; 35742; 35743; 35744; 3574. More particularly, it can be derived, without to be limited thereto, from the strains of Montreal or Pasteur Institute. For example, BCG can be PACIS BCG or TICE BCG. PACIS BCG is derived from Montreal strain which originates from a BCG culture given to Dr. Armand Frappier by Dr. C. Guérin in 1937. This strain was maintained by passaging until 1973. PACIS has also been known as "the Armand-Frappier strain of BCG".The TICE strain was developed at the University of Illinois from a strain originated at the Pasteur Institute. This strain was maintained by continuous passage for more than 20 years. For example, BCG can be prepared by the following process. BCG is grown on glycerinized potato medium followed by further passages on Sauton medium. After harvesting by filtration, BCG are resuspended in a 15% (w/v) lactose solution, filled into container, and preferably lyophilized.

Further mycobacterial antigen compositions that can use in accordance with the invention may comprise a major mycobacterial antigen or a recombinant mycobacterial strains and DNA forms. For example, of major antigens that are targets of the immune response to infection by Mycobacteria have been reported in Kaufman, Immunol. Today 11: 129-136 (1990); Young, Ann. Rev. Immunol. 8: 401-420 (1990); Young et al., Academic Press Ltd., London, pp. 1-35,

1990; Young et al, Mol. Microbiol. 6: 133-145 (1992)]. Recombinant BCG vaccine vehicles have been proposed (Snapper et al., PNAS. USA. 85: 6987-6991 (1988); Husson et al., J. Bacteriol. 172: 519-524 (1990); Martin et al., Nature 345: 739-743 (1990); Snapper et al, Mol. Microbiol. 4: 1911-1919 (1990); Aldovini and Young, Nature 351: 479-482 (1991); Jacobs et al, Methods Enzymology 204:537-555 (1991); Lee et al, PNAS 88: 3111-3115 (1991); Stover et al., Nature 351:456-460 (1991); Winter et al., Gene 109: 47-54 (1991); Donnelly-Wu et al., Mol. Microbiol. 7: 407-417 (1993)). Examples of recombinant DNA forms and strains of Mycobacteria are provided in U.S. Patents No. 5,866,403 describing the production and uses of homologously recombinant slow growing Mycobacteria; No. 5,854,055 describing recombinant Mycobacteria vaccine vehicles capable of expressing a foreign DNA of interest; No. 5,840,855 describing Mycobacterial recombinants and peptides encoded by the genome of Mycobacterium tuberculosis for use as vectors and protein expression; No. 5,830,475 presenting recombinant Mycobacterial vaccines which express a heterologous DNA encoding a protein or polypeptide product such as a cytokine; No. 5,807,723 offering a homologously recombinant slow growing Mycobacteria and methods of manipulating the genomic, DNA of slow growing Mycobacterial species; No, 5,504,005 describing a recombinant Mycobacterial vaccine capable of expressing a foreign DNA of interest against which an immune response is desired; No. 5.591,632 presenting a recombinant BCG -Mycobacteria expressing heterologous DNA encoding a polypeptide or protein for initiating an immune response; and No. 5.776,465 describing recombinant Mycobacterial vaccines, particularly a recombinant M. bovis BCG species which expresses heterologous DNA. Each of the references listed in this paragraph are incorporated herein by reference.

For the treatment of bladder cancer, the pharmaceutical composition comprising the Mycobacterium antigen, and optionally the γδT cell activator, can be administered locally at the disease site (the bladder) via intravesical treatment as following. The subject should not drink fluids for 4 hours before treatment and should empty its bladder prior administration of the pharmaceutical composition. The pharmaceutical composition is instilled into the bladder slowly (e.g., by gravity flow) via a catheter. The pharmaceutical composition is retained in the bladder for about two hours and then voided. During the period of treatment, more particularly during the first hour after the instillation, the subject should lie for 15 minutes each in the prone and suspine positions and also on each side, to maximize surface exposure to the pharmaceutical composition.Preferably, the treatment cycle consists of one intravesicular instillation per week for six weeks. Thereafter, the treatment can be continued at monthly intervals for 6-12 months. Optionally, the treated subjects can be evaluated, for example at 3, 6, and/or 9 months, after the

treatment. The evaluation can be performed by cytoscopy, cytology and/or biopsy. Patients may also continue to be treated as maintenance therapy.

A preferred composition comprising a mycobacterial antigen for use in accordance with the invention is Immucyst® (Bacillus Calmette-Guérin (BCG), substrain Connaught) available from Aventis Pasteur. As reproduced from the ImmuCyst® drug label, the product is made from a culture of an attenuated strain of living bovine tubercle bacillus Mycobacterium bovis. The bacilli are lyophilized (freeze-dried) and are viable upon reconstitution. When plated on culture media, the progenitor of each colony is termed a "colony-forming unit" (CFU); each CFU is composed of at least one viable bacillus and may comprise several bacilli, some of which may be viable and some non-viable. Each vial contains 81 mg (dry weight) of BCG and 5% w/v monosodium glutamate. Each vial of ImmuCyst® is reconstituted with the accompanying diluent (3.0 mL), which consists of approximately 0.85% w/v sodium chloride, 0.025% w/v Tween 80, 0.06% w/v sodium dihydrogen phosphate and 0.25% w/v disodium hydrogen phosphate. The product and the diluent contain no preservative. One dose consists of one 81 mg vial of reconstituted material further diluted in 50 mL sterile, preservative-free saline. The reconstituted dose contains $10.5 \pm 8.7 \times 10^8$ colony forming units (CFU) over the course of its shelf-life.

When administered intravesically as a cancer therapy, BCG promotes a local acute inflammatory and sub-acute granulomatous reaction with histiocytic and leukocytic infiltration in the urothelium and lamina propria of the urinary bladder. The local inflammatory effects are associated with an elimination or reduction of superficial cancerous lesions of the urinary bladder.

ImmuCyst® is commercialised for treatment of superficial transitional cell carcinoma (TCC) of the urinary bladder, including carcinoma in situ (CIS), papillary tumors limited to the mucosa (stage Ta), papillary tumors involving the lamina propria but not the muscle layer of the bladder (stage T1), or any combination thereof. ImmuCyst® is indicated for the treatment and prophylaxis of primary or recurrent carcinoma in-situ (CIS) of the urinary bladder, and for prophylaxis following TUR of primary or recurrent stage Ta and/or T1 papillary tumors.

ImmuCyst® is preferably dosed and administered according to the manufacturer's instructions as follows. Intravesical treatment of the urinary bladder using ImmuCyst® is recommended to begin between 7 to 14 days after biopsy or transurethral resection. The induction treatment comprises 6 weekly intravesical treatments with ImmuCyst®, each treatment dose comprising one 81 mg vial of ImmuCyst®. After a 6-week pause, another dose should be given

intravesically once weekly for 1-3 weeks. Three weekly doses should definitely be given to patients who still have evidence of bladder cancer. Clinical studies have demonstrated that the 3 doses given at 3 months significantly increased the complete response rate from 73% to 87% at 6 months. Maintenance therapy following induction is recommended. This consists of 1-3 weekly treatments at 6 months following the initiation of treatment, and then every 6 months thereafter until 36 months.

Each dose (1 reconstituted vial) is further diluted in an additional 50 mL of sterile, preservative-free saline for a total of 53 mL (see reconstitution instructions below). A urethral catheter is inserted into the bladder under aseptic conditions, the bladder is drained, and then 53 mL suspension of ImmuCyst® is instilled slowly by gravity, following which the catheter is withdrawn. The patient retains the suspension for as long as possible for a total of up to two hours. During the first 15 minutes following instillation, the patient should lie prone. Thereafter, the patient is then allowed to be up. At the end of 2 hours, all patients should void in a seated position for environmental safety reasons. Patients should be instructed to maintain adequate hydration.

Another preferred mycobacterial antigen-containing composition made from a culture of an attenuated strain of living BCG is TICE™ BCG (Organon Teknika Corp.). TICE™ is commercialised for the treatment of carcinoma in situ of the bladder. TICE™ can be administered according to the manufacturer's instructions as follows, recommending that the product is administered 7-14 days after bladder biopsy. The reconstituted TICE™ is installed into the bladder by gravity flow using a catheter, is maintained in the bladder for two hours and then voided. While the BCG is in the bladder, the patient should be repositioned from the left side to the right side and the back side to the abdomen every 15 minutes in order to maximize surface exposure to the agent. A standard treatment of TICE consists of one intravesicular instillation per week for six weeks. The schedule may be repeated once if tumor remission has not been achieved. Thereafter, TICE™ administration can be continued at monthly intervals for 6-12 months.

Another preferred mycobacterial antigen-containing composition made from a culture of an attenuated strain of living BCG is Pacis™ (Shire Biologics, Sainte-Foy, Quebec, Canada and Urocor, Inc., Oklahoma USA). Pacis™ is supplied as a single dose ampule of 120mg (semi-dry weight) lyophilised BCG (2.4 to $12x10^8$ C.F.U. per ampule). Pacis™ is commercialised for the treatment of bladder cancer and treatment. Pacis™ can be administered in accordance with the

manufacturer's instructions at a single dose of 120mg according to the same methods as TICE™ once weekly for six-weeks, which cycle may be repeated if tumor remission has not been achieved.

Another preferred mycobacterial antigen-containing composition is BCG-Medac, made from a culture of an attenuated strain of living BCG, strain RIVM derived from strain 1173-P2 (medac, Hamburg, Germany). BCG-medac is commercialised for the treatment of bladder cancer and treatment can be carried out in accordance with the manufacturer's instructions as follows. The reconstituted dose of BCG-medac contains $2x10^8$ - 3 x 10^0 colony forming units (CFU) of attenuated BCG and is installed into the bladder 2 to 3 weeks after transurethral resection using a single use catheter under slight pressure. A standard treatment of BCG-medac consists of one intravesicular instillation per week for six weeks. The schedule may be repeated if tumor remission has not been achieved. After a treatment-free period of 4 weeks, BCG-medac administration can be continued at monthly intervals for a 12 month period. Alternatively, BCG-medac can be given for six weeks followed by weekly injections for three consecutive weeks in months 3, 6, 12, 18, 24, 30 and 36.

An efficient dose of an attenuated BCG strain according the present invention preferably comprises about 0.1 to 50 x 10^8 colony forming units, more preferably 1 to 15 10^8 colony forming units.

Yet another preferred mycobacterial antigen-containing composition is the product referred to as SRL172 (SR Pharma, London, U.K.), a killed *Mycobacterium vaccae* suspension also described in PCT application no. WO85/03639, the disclosure of which is incorporated herein by reference.

Preparations of bacterial origin, including, but not limited to, preparations from Mycobacterium species, have been used to treat cancers (U.S. Pat. No. 4,503,048, the disclosure of which is incorporated herein by reference). One example is REGRESSIN™, a non-viable mycobacterial cell wall extract (MCWE) formulated as a mineral oil emulsion (Bioniche, Inc. London, Ontario, Canada), which has been shown to reduce cancer burden in bladder cancers (Kadhim et al. Journal of Urology 149:A255, 1996; Morales et al. Journal of Urology 157:A214, 1997). MCWE is composed primarily of peptidoglycan and glycolipid (Chin et al. Journal of Urology 156:1189-1193, 1996) and contain N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide) and mycolic acid derivatives. Both muramyl dipeptide and mycolic acid derivatives stimulate the immune system by activation of macrophage and monocyte mediated reactions

(Mallick et al. Comparative Immunology and Microbiology of Infectious Diseases 8:55-63, 1985; Teware et al. Veterinary Parasitology 62:223-230, 1996).

Another preparation that can be used in accordance with the invention is a Mycobacterium cell wall composition, preferably deproteinized and delipidated and optionally complexed to DNA. Such a composition comprising a Mycobacterium phlei deoxyribonucleic acid (M-DNA)-Mycobacterium phlei cell wall complex (MCC) is provided in US Patent no 6,329,347, the disclosure of which is incorporated herein by reference.

Another preferred preparation is a DNA-rich fraction extracted and purified from mycobacterium bovis BCG referred to as MY-1. MY-1 is described in Fujeida et al, (1999) Am. J. Respir. Crit. Care med. 160: 2056-2061, and the preparation of MY-1 is described in Tokunaga et al, (1984) J. Natl. Cancer Inst. 72:955-962, both of which disclosures are incorporated herein by reference.

γδT cell activators

The term "γδT cell activator" designates a molecule, preferably artificially produced, which can activate γδT lymphocytes. It is more preferably a ligand of the T receptor of γδT lymphocytes. The activator may by of various nature, such as a peptide, lipid, small molecule, etc. It may be a purified or otherwise artificially produced (e.g., by chemical synthesis, or by microbiological process) endogenous ligand, or a fragment or derivative thereof, or an antibody having substantially the same antigenic specificity.

The γδ T cell activator preferably increases the biological activity or causes the proliferation of γδ T cells, preferably increasing the activation of γδ T cells, particularly increasing cytokine secretion from γδ T cells or increasing the cytolytic activity of γδ T cells, with or without also stimulating the proliferation or expansion of γδ T cells. Accordingly, the γδ T cell activator is administered in an amount and under conditions sufficient to increase the activity γδ T cells in a subject, preferably in an amount and under conditions sufficient to increase cytokine secretion by γδ T cells and/or to increase the cytolytic activity of γδ T cells. Cytokine secretion and cytolytic activity can be assessed using any appropriate in vitro assay.

In any exemplary assay, cytokine secretion can be determined according to the methods described in Espinosa et al. (J. Biol. Chem., 2001, Vol. 276, Issue 21, 18337-18344), describing

measurement of TNF-α release in a bioassay using TNF-α-sensitive cells. Briefly, 10^4 γδT cells/well were incubated with stimulus plus 25 units of IL2/well in 100 µl of culture medium during 24 h at 37 °C. Then, 50 µl of supernatant were added to 50 µl of WEHI cells plated at 3×10^4 cells/well in culture medium plus actinomycin D (2 µg/ml) and LiCl (40 mM) and incubated for 20 h at 37 °C. Viability of the TNF-α-sensitive cells and measured with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. 50 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma; 2.5 mg/ml in phosphate-buffered saline) per well were added, and after 4 h of incubation at 37 °C, 50 µl of solubilization buffer (20% SDS, 66% dimethyl formamide, pH 4.7) were added, and absorbance (570 nm) was measured. Levels of TNF-α release were then calculated from a standard curve obtained using purified human rTNF-α (PeproTech, Inc., Rocky Hill, NJ). Interferon-γ released by activated T cells was measured by a sandwich enzyme-linked immunosorbent assay. 5×10^4 γδT cells/well were incubated with stimulus plus 25 units of IL2/well in 100 µl of culture medium during 24 h at 37 °C. Then, 50 µl of supernatant were harvested for enzyme-linked immunosorbent assay using mouse monoclonal antibodies (BIOSOURCE, Camarillo, CA).

A preferred assay for cytolytic activity is a ^{51}Cr release assay. In exemplary assays, the cytolytic activity of γδ T cells is measured against autologous normal and tumor target cell lines, or control sensitive target cell lines such as Daudi and control resistant target cell line such as Raji in 4h ^{51}Cr release assay. In a specific example, target cells were used in amounts of $2x10^3$ cells/well and labeled with 100µCi ^{51}Cr for 60 minutes. Effector/Target (E/T) ratio ranged from 30: 1 to 3.75: 1. Specific lysis (expressed as percentage) is calculated using the standard formula [(experimental-spontaneous release / total-spontaneous release) x100].

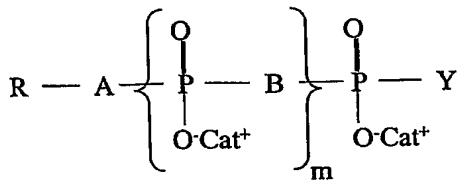
As discussed, the methods of the invention can generally be carried out with any γδ T cell activator that is capable of stimulating γδ T cell activity. This stimulation can be by direct effect on γδ T cells as discussed below using compounds that can stimulate γδ T cells in a pure γδ T cell culture, or the stimulation can be by an indirect mechanism, such as treatment with pharmacological agents such as statins which prevent biosynthesis of the γδ T cell-stimulating compound isopentenyl pyrophosphate (IPP) or aminobisphosphonates which lead to IPP accumulation (such as, see below). Preferably, however, a γδ T cell activator is a compound capable of regulating the activity of a γδ T cell in a population of γδ T cell clones in culture. The γδ T cell activator is capable of regulating the activity of a γδ T cell population of γδ T cell clones at millimolar concentration, preferably when the γδ T cell activator is present in culture at a concentration of less than 100 mM. Optionally a γδ T cell activator is capable of regulating

the activity of a γδ T cell in a population of γδ T cell clones at millimolar concentration, preferably when the γδ T cell activator is present in culture at a concentration of less than 10 mM, or more preferably less than 1 mM. Regulating the activity of a γδ T cell can be assessed by any suitable means, preferably by assessing cytokine secretion, most preferably TNF-α secretion as described herein. Methods for obtaining a population of pure γδ T cell clones is described in Davodeau et al, (1993) and Moreau et al, (1986), the disclosures of which are incorporated herein by reference. Preferably the activator is capable of causing at least a 20%, 50% or greater increase in the number of γδ T cells in culture, or more preferably at least a 2-fold increase in the number of γδ T cells in culture.

In one embodiment, the activator may be a synthetic chemical compound capable of selectively activating Vγ9Vδ2 T lymphocytes. Selective activation of Vγ9Vδ2 T lymphocytes indicates that the compound has a selective action towards specific cell populations, preferably increasing activation of Vγ9Vδ2 T cells at a greater rate or to a greater degree than other T cell types such as Vδ1 T cells, or not substantially not activation other T cell types. Such selectivity can be assessed in vitro T cell activation assays. Such selectivity, as disclosed in the present application, suggests that preferred compounds can cause a selective or targeted activation of the proliferation or biological activity of Vγ9Vδ2 T lymphocytes.

In a preferred embodiment, said γδT cell activator is a compound of the formula I, especially a γδ T cell activator according to formulas I to XVII, especially γδ T cell activator selected from the group consisting of BrHPP, CBrHPP, HDMAPP HDMAPP and epoxPP. However, it will appreciated that a number of less potent γδ T cell activators are available and may be used in accordance with the invention. For example, in one variant, aminobiphosphonate compounds such as pamidronate (Novartis, Nuernberg, Germany) and zoledronate may be used. Other γδ T cell activators for use in the present invention are phosphoantigens disclosed in WO95/20673, isopentenyl pyrophosphate (IPP) (US5,639,653), as well as alkylamines (such as ethylamine, iso-propyulamine, n-propylamine, n-butylamine and iso-butylamine, for instance). Isobutyl amine and 3-aminopropyl phosphonic acid are obtained from Aldrich (Chicago, IL).

Examples of preferred γδ T cell activators according to the present invention comprise the compounds of formula (I) :



Formula (I)

wherein Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including proton);

m is an integer from 1 to 3;

B is O, NH, or any group capable to be hydrolyzed;

Y = O^-Cat^+, a C_1-C_3 alkyl group, a group -A-R, or a radical selected from the group consisting of a nucleoside, an oligonucleotide, a nucleic acid, an amino acid, a peptide, a protein, a monosaccharide, an oligosaccharide, a polysaccharide, a fatty acid, a simple lipid, a complex lipid, a folic acid, a tetrahydrofolic acid, a phosphoric acid, an inositol, a vitamin, a co-enzyme, a flavonoid, an aldehyde, an epoxyde and a halohydrin;

A is O, NH, CHF, CF_2 or CH_2; and,

R is a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C_1-C_{50} hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkylenyl, or an alkynyl, preferably an alkyl or an alkylene, which can be substituted by one or several substituents selected from the group consisting of : an alkyl, an alkylenyl, an alkynyl, an epoxyalkyl, an aryl, an heterocycle, an alkoxy, an acyl, an alcohol, a carboxylic group (-COOH), an ester, an amine, an amino group ($-NH_2$), an amide ($-CONH_2$), an imine, a nitrile, an hydroxyl (-OH), a aldehyde group (-CHO), an halogen, an halogenoalkyl, a thiol (-SH), a thioalkyl, a sulfone, a sulfoxide, and a combination thereof.

In a particular embodiment, the substituents as defined above are substituted by at least one of the substituents as specified above.

Preferably, the substituents are selected from the group consisting of : an (C_1-C_6)alkyl, an (C_2-C_6)alkylenyl, an (C_2-C_6)alkynyl, an (C_2-C_6)epoxyalkyl, an aryl, an heterocycle, an (C_1-C_6)alkoxy, an (C_2-C_6)acyl, an (C_1-C_6)alcohol, a carboxylic group (-COOH), an (C_2-C_6)ester, an (C_1-C_6)amine, an amino group ($-NH_2$), an amide ($-CONH_2$), an (C_1-C_6)imine, a nitrile, an hydroxyl (-OH), a aldehyde group (-CHO), an halogen, an (C_1-C_6)halogenoalkyl, a thiol (-SH), a (C_1-C_6)thioalkyl, a (C_1-C_6)sulfone, a (C_1-C_6)sulfoxide, and a combination thereof.

More preferably, the substituents are selected from the group consisting of : an (C_1-C_6)alkyl, an (C_2-C_6)epoxyalkyl, an (C_2-C_6)alkylenyl, an (C_1-C_6)alkoxy, an (C_2-C_6)acyl, an (C_1-C_6)alcohol, an

(C_2-C_6)ester, an (C_1-C_6)amine, an (C_1-C_6)imine, an hydroxyl, a aldehyde group, an halogen, an (C_1-C_6)halogenoalkyl, and a combination thereof.

Still more preferably, the substituents are selected from the group consisting of : an (C_3-C_6)epoxyalkyl, an (C_1-C_3)alkoxy, an (C_2-C_3)acyl, an (C_1-C_3)alcohol, an (C_2-C_3)ester, an (C_1-C_3)amine, an (C_1-C_3)imine, an hydroxyl, an halogen, an (C_1-C_3)halogenoalkyl, and a combination thereof. and a combination thereof. Preferably, R is a (C_3-C_{25})hydrocarbon group, more preferably a (C_5-C_{10})hydrocarbon group.

In the context of the present invention, the term "alkyl" more specifically means a group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heneicosyl, docosyl and the other isomeric forms thereof. (C_1-C_6)alkyl more specifically means methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, pentyl, hexyl and the other isomeric forms thereof. (C_1-C_3)alkyl more specifically means methyl, ethyl, propyl, or isopropyl.

The term "alkenyl" refers to an alkyl group defined hereinabove having at least one unsaturated ethylene bond and the term "alkynyl" refers to an alkyl group defined hereinabove having at least one unsaturated acetylene bond. (C_2-C_6)alkylene includes a ethenyl, a propenyl (1-propenyl or 2-propenyl), a 1- or 2- methylpropenyl, a butenyl (1-butenyl, 2-butenyl, or 3-butenyl), a methylbutenyl, a 2-ethylpropenyl, a pentenyl (1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl), an hexenyl (1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl), and the other isomeric forms thereof. (C_2-C_6)alkynyl includes ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl, 1-pentynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl and the other isomeric forms thereof.

The term "epoxyalkyl" refers to an alkyl group defined hereinabove having an epoxide group. More particularly, (C_2-C_6)epoxyalkyl includes epoxyethyl, epoxypropyl, epoxybutyl, epoxypentyl, epoxyhexyl and the other isomeric forms thereof. (C_2-C_3)epoxyalkyl includes epoxyethyl and epoxypropyl.

The "aryl" groups are mono-, bi- or tri-cyclic aromatic hydrocarbons having from 6 to 18 carbon atoms. Examples include a phenyl, α-naphthyl, β-naphthyl or anthracenyl group, in particular.

"Heterocycle" groups are groups containing 5 to 18 rings comprising one or more heteroatoms, preferably 1 to 5 endocyclic heteroatoms. They may be mono-, bi- or tri-cyclic. They may be aromatic or not. Preferably, and more specifically for R_5, they are aromatic heterocycles. Examples of aromatic heterocycles include pyridine, pyridazine, pyrimidine, pyrazine, furan, thiophene, pyrrole, oxazole, thiazole, isothiazole, imidazole, pyrazole, oxadiazole, triazole, thiadiazole and triazine groups. Examples of bicycles include in particular quinoline, isoquinoline and quinazoline groups (for two 6-membered rings) and indole, benzimidazole, benzoxazole, benzothiazole and indazole (for a 6-membered ring and a 5-membered ring). Nonaromatic heterocycles comprise in particular piperazine, piperidine, etc.

"Alkoxy" groups correspond to the alkyl groups defined hereinabove bonded to the molecule by an -O- (ether) bond. $(C_1\text{-}C_6)$alkoxy includes methoxy, ethoxy, propyloxy, butyloxy, pentyloxy, hexyloxy and the other isomeric forms thereof. $(C_1\text{-}C_3)$alkoxy includes methoxy, ethoxy, propyloxy, and isopropyloxy.

"Alcyl" groups correspond to the alkyl groups defined hereinabove bonded to the molecule by an -CO- (carbonyl) group. $(C_2\text{-}C_6)$acyl includes acetyl, propylacyl, butylacyl, pentylacyl, hexylacyl and the other isomeric forms thereof. $(C_2\text{-}C_3)$acyl includes acetyl, propylacyl and isopropylacyl.

"Alcohol" groups correspond to the alkyl groups defined hereinabove containing at least one hydroxyl group. Alcohol can be primary, secondary or tertiary. $(C_1\text{-}C_6)$alcohol includes methanol, ethanol, propanol, butanol, pentanol, hexanol and the other isomeric forms thereof. $(C_1\text{-}C_3)$alcohol includes methanol, ethanol, propanol and isopropanol.

"Ester" groups correspond to the alkyl groups defined hereinabove bonded to the molecule by an -COO- (ester) bond. $(C_2\text{-}C_6)$ester includes methylester, ethylester, propylester, butylester, pentylester and the other isomeric forms thereof. $(C_2\text{-}C_3)$ester includes methylester and ethylester.

"Amine" groups correspond to the alkyl groups defined hereinabove bonded to the molecule by an –N- (amine) bond. $(C_1\text{-}C_6)$amine includes methylamine, ethylamine, propylamine, butylamine, pentylamine, hexylamine and the other isomeric forms thereof. $(C_1\text{-}C_3)$amine includes methylamine, ethylamine, and propylamine.

"Imine" groups correspond to the alkyl groups defined hereinabove having a (-C=N-) bond. (C_1-C_6)imine includes methylimine, ethylimine, propylimine, butylimine, pentylimine, hexylimine and the other isomeric forms thereof. (C_1-C_3)imine includes methylimine, ethylimine, and propylimine.

The halogen can be Cl, Br, I, or F, more preferably Br or F.

"Halogenoalkyl" groups correspond to the alkyl groups defined hereinabove having at least one halogen. The groups can be monohalogenated or polyhalogenated containing the same or different halogen atoms. For example, the group can be an trifluoroalkyl (CF_3-R). (C_1-C_6)halogenoalkyl includes halogenomethyl, halogenoethyl, halogenopropyl, halogenobutyl, halogenopentyl, halogenohexyl and the other isomeric forms thereof. (C_1-C_3)halogenoalkyl includes halogenomethyl, halogenoethyl, and halogenopropyl.

"Thioalkyl" groups correspond to the alkyl groups defined hereinabove bonded to the molecule by an -S- (thioether) bond. (C_1-C_6)thioalkyl includes thiomethyl, thioethyl, thiopropyl, thiobutyl, thiopentyl, thiohexyl and the other isomeric forms thereof. (C_1-C_3)thioalkyl includes thiomethyl, thioethyl, and thiopropyl.

"Sulfone" groups correspond to the alkyl groups defined hereinabove bonded to the molecule by an -SOO- (sulfone) bond. (C_1-C_6)sulfone includes methylsulfone, ethylsulfone, propylsulfone, butylsulfone, pentylsulfone, hexylsulfone and the other isomeric forms thereof. (C_1-C_3)sulfone includes methylsulfone, ethylsulfone and propylsulfone.

"Sulfoxyde" groups correspond to the alkyl groups defined hereinabove bonded to the molecule by an -SO- (sulfoxide) group. (C_1-C_6)sulfoxide includes methylsulfoxide, ethylsulfoxide, propylsulfoxide, butylsulfoxide, pentylsulfoxide, hexylsulfoxide and the other isomeric forms thereof. (C_1-C_3)sulfoxide includes methylsulfoxide, ethylsulfoxide, propylsulfoxide and isopropylsulfoxide.

"Heteroatom" denotes N, S, or O.

"Nucleoside" includes adenosine, thymine, uridine, cytidine and guanosine.

In a particular embodiment, the hydrocarbon group is a cycloalkylenyl such as a cyclopentadiene or a phenyl, or an heterocycle such as a furan, a pyrrole, a thiophene, a

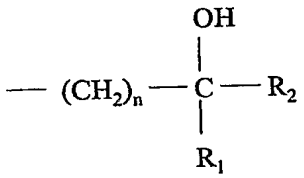
thiazole, an imidazole, a triazole, a pyridine, a pyrimidine, a pyrane, or a pyrazine. Preferably, the cycloalkylenyl or the heterocycle is selected from the group consisting of a cyclopentadiene, a pyrrole or an imidazole. In a preferred embodiment, the cycloalkylenyl or the heterocycle is sustituted by an alcohol. Preferably, said alcohol is a (C_1-C_3)alcohol.

In an other embodiment, the hydrocarbon group is an alkylenyl with one or several double bonds. Preferably, the alkylenyl group has one double bond. Preferably, the alkylenyl group is a (C_3-C_{10})alkylenyl group, more preferably a (C_4-C_7)alkylenyl group. Preferably, said alkylenyl group is substituted by at least one functional group. More preferably, the functional group is selected from the group consisting of an hydroxy, an (C_1-C_3)alkoxy, an aldehyde, an (C_2-C_3)acyl, or an (C_2-C_3)ester. In a more preferred embodiment, the hydrocarbon group is butenyl substituted by a group $-CH_2OH$. Optionally, said alkenyl group can be the isoform trans (E) or cis (Z), more preferably a trans isoform (E). In a most preferred embodiment, the alkylenyl group is the (E)-4-hydroxy-3-methyl-2-butenyl. In an other preferred embodiment, the alkylenyl group group is an isopentenyl, an dimethylallyl or an hydroxydimethylallyl.

In an additional embodiment, the hydrocarbon group is an alkyl group substituted by an acyl. More preferably, the hydrocarbon group is an (C_4-C_7)alkyl group substituted by an (C_1-C_3)acyl.

In a further preferred embodiment, R is selected from the group consisting of :

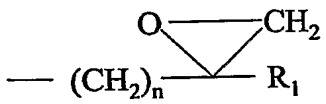
1)



wherein n is an integer from 2 to 20, R_1 is a (C_1-C_3)alkyl group, and R_2 is an halogenated (C_1-C_3)alkyl, a (C_1-C_3)alkoxy-(C_1-C_3)alkyl, an halogenated (C_2-C_3)acyl or a (C_1-C_3)alkoxy-(C_2-C_3)acyl. Preferably, R_1 is a methyl or ethyl group, and R_2 is an halogenated methyl ($-CH_2$-X, X being an halogen), an halogenated (C_2-C_3)acetyl, or (C_1-C_3)alkoxy- acetyl. The halogenated methyl or acetyl can be mono-, di-, or tri-halogenated. Preferably, n is an integer from 2 to 10, or from 2 to 5. In a more preferred embodiment, n is 2. In a most preferred embodiment, n is 2, R_1 is a methyl and R_2 is an halogenated methyl, more preferably a monohalogenated methyl,. still more preferably a bromide methyl. In a particularly preferred embodiment, n is 2, R_1 is a

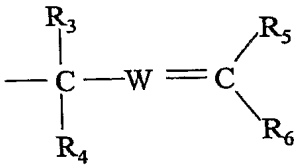
methyl, R2 is a methyl bromide. In a most preferred embodiment, R is 3-(bromomethyl)-3-butanol-1-yl.

2)



wherein n is an integer from 2 to 20, and R_1 is a methyl or ethyl group. Preferably, n is an integer from 2 to 10, or from 2 to 5. In a more preferred embodiment, n is 2 and R1 is a methyl.

3)



wherein R_3, R_4, and R_5 , identical or different, are a hydrogen or $(C_1$-$C_3)$alkyl group, W is –CH- or –N-, and R_6 is an $(C_2$-$C_3)$acyl, an aldehyde, an $(C_1$-$C_3)$alcohol, or an $(C_2$-$C_3)$ester. More preferably, R_3 and R_5 are a methyl and R_4 is a hydrogen. More preferably, R_6 is -CH_2-OH, -CHO, -CO-CH_3 or -CO-OCH_3. Optionally, the double-bond between W and C is in conformation trans (E) or cis (Z). More preferably, the double-bond between W and C is in conformation trans (E).

The group Y can allow to design a prodrug. Therefore, Y is enzymolabile group which can be cleaved in particular regions of the subject. The group Y can also be targeting group. In a preferred embodiment, Y is O$^-$Cat+, a group -A-R, or a radical selected from the group consisting of a nucleoside, a monosaccharide, an epoxyde and a halohydrin. Preferably, Y is an enzymolabile group. Preferably, Y is O$^-$Cat+, a group -A-R, or a nucleoside. In a first preferred embodiment, Y is O$^-$Cat+. In a second preferred embodiment, Y is a nucleoside.

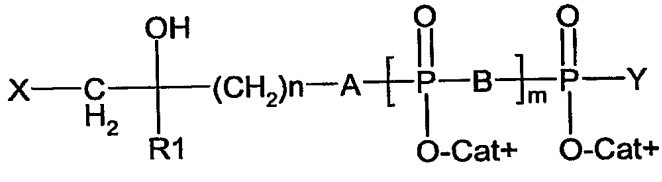
In a preferred embodiment, Cat^+ is H^+, Na^+, NH_4^+, K^+, Li^+, $(CH_3CH_2)_3NH^+$.

In a preferred embodiment, A is O, CHF, CF_2 or CH_2. More preferably, A is O or CH_2.

In a preferred embodiment, B is O or NH. More preferably, B is O.

In a preferred embodiment, m is 1 or 2. More preferably, m is 1.

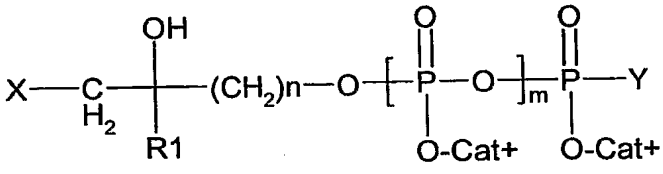
In one particular embodiment, synthetic γδT cell activators comprise the compounds of formula (II):



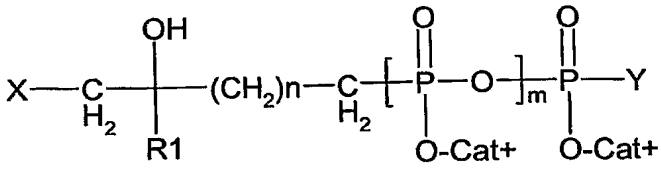
(II)

in which X is an halogen (preferably selected from I, Br and Cl), B is O or NH, m is an integer from 1 to 3, R1 is a methyl or ethyl group, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), and n is an integer from 2 to 20, A is O, NH, CHF, CF_2 or CH_2, and Y is O^-Cat+, a nucleoside, or a radical –A-R, wherein R is selected from the group of 1), 2) or 3). Preferably, Y is O^-Cat+, or a nucleoside. More preferably, Y is O^-Cat+. Preferably, R1 is a methyl. Preferably, A is O or CH_2. More preferably, A is O. Preferably, n is 2. Preferably, X is a bromide. Preferably, B is O. Preferably, m is 1 or 2. More preferably, m is 1.

For example, synthetic γδT cell activators comprise the compounds of formula (III) or (IV) :



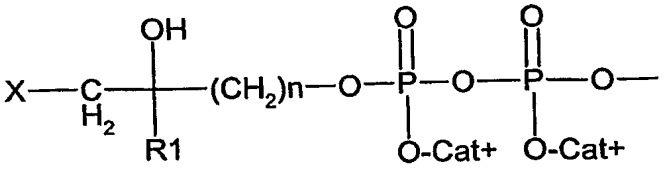
(III)



(IV)

wherein X, R1, n, m and Y have the aforementioned meaning.

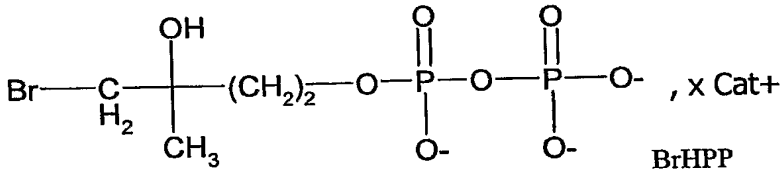
In one preferred embodiment, synthetic γδT cell activators comprise the compounds of formula (V):



(V)

in which X is an halogen (preferably selected from I, Br and Cl), R1 is a methyl or ethyl group, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), and n is an integer from 2 to 20. Preferably, R1 is a methyl. Preferably, n is 2. Preferably, X is a bromide.

In a most preferred embodiment, synthetic γδT cell activators comprise the compound of formula (VI):

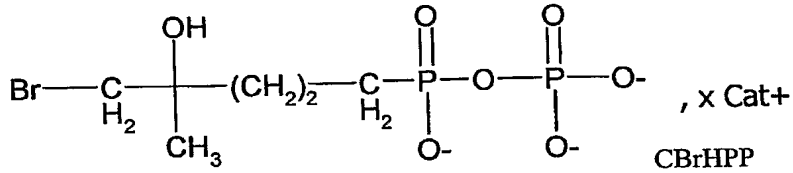


(VI)

BrHPP

Preferably x Cat+ is 1 or 2 Na^+.

In an other most preferred embodiment, synthetic γδT cell activators comprise the compound of formula (VII):

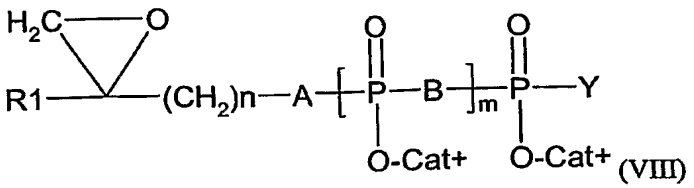


(VII)

CBrHPP

Preferably x Cat+ is 1 or 2 Na^+.

In one particular embodiment, synthetic γδT cell activators comprise the compounds of formula (VIII):

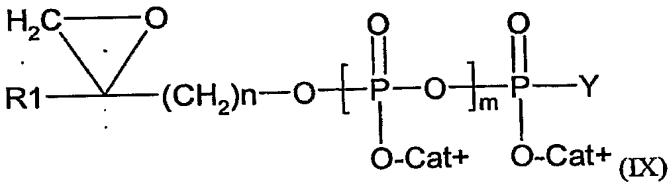


(VIII)

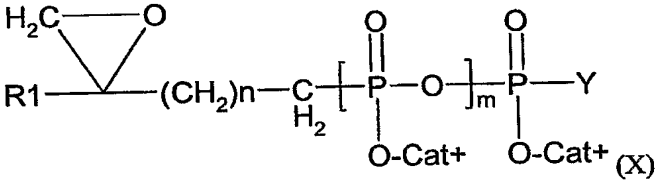
in which R1 is a methyl or ethyl group, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), B is O or NH, m is an integer from 1 to 3,

and n is an integer from 2 to 20, A is O, NH, CHF, CF_2 or CH_2, and Y is O^-Cat+, a nucleoside, or a radical –A-R, wherein R is selected from the group of 1), 2) or 3). Preferably, Y is O^-Cat+, or a nucleoside. More preferably, Y is O^-Cat+. Preferably, R1 is a methyl. Preferably, A is O or CH_2. More preferably, A is O. Preferably, n is 2. Preferably, B is O. Preferably, m is 1 or 2. More preferably, m is 1.

For example, synthetic γδT cell activators comprise the compounds of formula (IX) or (X) :



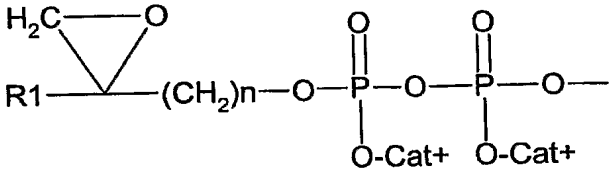
(IX)



(X)

wherein R1, n, m and Y have the above mentioned meaning.

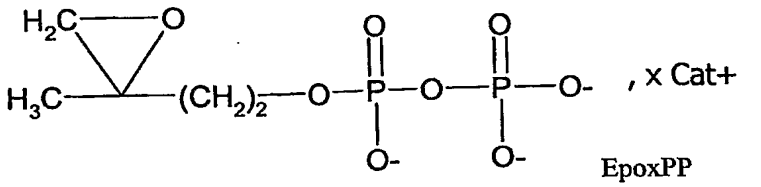
In one preferred embodiment, synthetic γδT cell activators comprise the compounds of formula (XI):



(XI)

in which R1 is a methyl or ethyl group, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), and n is an integer from 2 to 20. Preferably, R1 is a methyl. Preferably, n is 2.

In a most preferred embodiment, synthetic γδT cell activators comprise the compound of formula (XI):

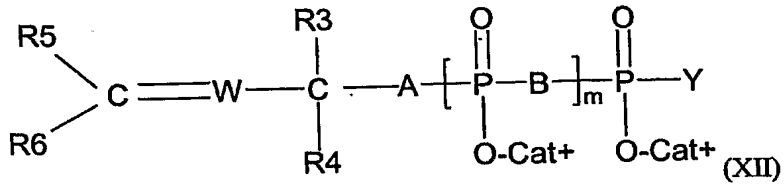


(XI)

EpoxPP

Preferably x Cat+ is 1 or 2 Na^+.

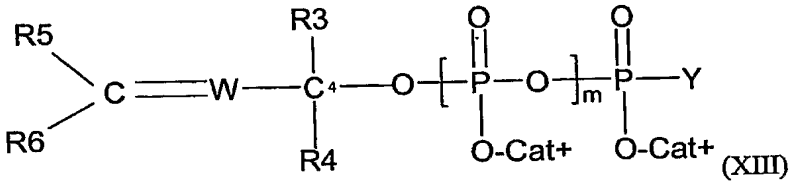
In one particular embodiment, synthetic γδT cell activators comprise the compounds of formula (XII):



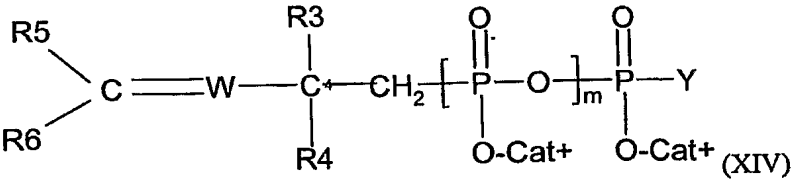
(XII)

in which R_3, R_4, and R_5 , identical or different, are a hydrogen or (C_1-C_3)alkyl group, W is –CH- or –N-, R_6 is an (C_2-C_3)acyl, an aldehyde, an (C_1-C_3)alcohol, or an (C_2-C_3)ester, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), B is O or NH, m is an integer from 1 to 3, A is O, NH, CHF, CF_2 or CH_2, and Y is O⁻Cat+, a nucleoside, or a radical –A-R, wherein R is selected from the group of 1), 2) or 3). Preferably, Y is O⁻Cat+, or a nucleoside. More preferably, Y is O⁻Cat+. Preferably, A is O or CH_2. More preferably, A is O. More preferably, R_3 and R_5 are a methyl and R_4 is a hydrogen. More preferably, R_6 is -CH_2-OH, -CHO, -CO-CH_3 or -CO-OCH_3. Preferably, B is O. Preferably, m is 1 or 2. More preferably, m is 1. Optionally, the double-bond between W and C is in conformation trans (E) or cis (Z). More preferably, the double-bond between W and C is in conformation trans (E).

For example, synthetic γδT cell activators comprise the compounds of formula (XIII) or (XIV) :



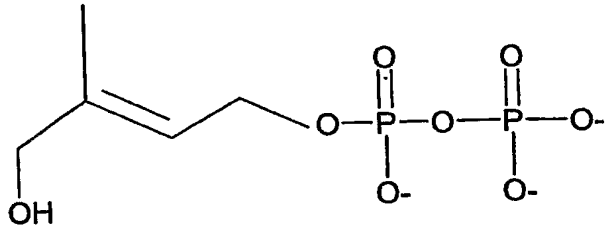
(XIII)



(XIV)

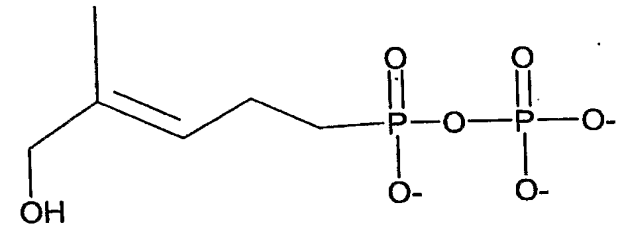
wherein R3, R4, R5, R6, W, m, and Y have the above mentionned meaning. Preferably, W is –CH-. Preferably, R3 and R4 are hydrogen. Preferably, R5 is a methyl. Preferably, R6 is –CH_2-OH.

In a most preferred embodiment, synthetic γδT cell activators comprise the compound of formula (XV):



(XV) HDMAPP

In an other most preferred embodiment, synthetic γδT cell activators comprise the compound of formula (XVI):



(XVI) CHDMAPP

Specific examples of compounds include : (E)1-pyrophosphonobuta-1,3-diene ; (E)1-pyrophosphonopenta-1,3-diene ; (E)1-pyrophosphono-4-methylpenta-1,3-diene ; (E,E)1-pyrophosphono-4,8-dimethylnona-1,3,7-triene ; (E,E,E)1-pyrophosphono-4,8,12-trimethyltrideca-1,3,7,11-tetraene ; (E,E)1-triphosphono-4,8-dimethylnona-1,3,7-triene ; 4-triphosphono-2-methylbutene ; α,β-di-[3-methylpent-3-enyl]-pyrophosphonate ; 1-pyrophosphono-3-methylbut-2-ene ; α,γ-di-[3-methylbut-2-enyl]-triphosphonate ; α,β-di-[3-methylbut-2-enyl]-pyrophosphonate ; allyl-pyrophosphonate ; allyl-triphosphonate ; α,γ-di-allyl-pyrophosphonate ; α,β-di-allyl-triphosphonate ; (E,E)4-[(5'-pyrophosphono-6'-methyl-penta-2',4'-dienyloxymethyl)-phenyl]-phenyl-methanone ; (E,E)4-[(5'-triphosphono-6'-methyl-penta-2',4'-dienyloxymethyl)-phenyl]-phenyl-methanone ; (E,E,E)[4-(9'-pyrophosphono-2',6'-dimethyl-nona-2',6',8'-trienyloxymethyl)-phenyl]-phenyl-methanone ; (E,E,E)[4-(9'-pyrophosphono-2',6',8'-trimethyl-nona-2',6',8'-trienyloxymethyl)-phenyl]-phenyl-methanone ; 5-pyrophosphono-2-methypentene ; 5-triphosphono-2-methypentene ; α,γ-di-[4-methylpent-4-enyl]-triphosphonate ; 5-pyrophosphono-2-methypent-2-ene ; 5-triphosphono-2-methypent-2-ene ; 9-pyrophosphono-2,6-dimethynona-2,6-diene ; 9-triphosphono-2,6-dimethynona-2,6-diene ; α,γ-di-[4,8-dimethylnona-2,6-dienyl]-triphosphonate ; 4-pyrophosphono-2-methybutene ; 4-methyl-2-oxa-pent-4-enyloxymethylpyrophosphate ; 4-methyl-2-oxa-pent-4-enyloxymethyltriphosphate ; α,β-di-[4-methyl-2-oxa-pent-4-enyloxymethyl]-pyrophosphate ; and α,γ-di-[4-methyl-2-oxa-pent-4-enyloxymethyl]-triphosphate.

In a particular embodiment, the γδT cell activator can be selected from the group consisting of : 3-(hálomethyl)-3-butanol-1-yl-diphosphate ; 3-(halomethyl)-3-pentanol-1-yl-diphsophate ; 4-(halomethyl)-4-pentanol-1-yl-diphosphate ; 4-(halomethyl)-4-hexanol-1-yl-diphosphate ; 5-(halomethyl)-5-hexanol-1-yl-diphosphate ; 5-(halomethyl)-5-heptanol-1-yl-diphosphate ; 6-(halomethyl)-6-heptanol-1-yl-diphosphate ; 6-(halomethyl)-6-octanol-1-yl-diphosphate ; 7-(halomethyl)-7-octanol-1-yl-diphosphate ; 7-(halomethyl)-7-nonanol-1-yl-diphosphate ; 8-(halomethyl)-8-nonanol-1-yl-diphosphate ; 8-(halomethyl)-8-decanol-1-yl-diphosphate ; 9-(halomethyl)-9-decanol-1-yl-diphosphate ; 9-(halomethyl)-9-undecanol-1-yl-diphosphate ; 10-(halomethyl)-10-undecanol-1-yl-diphosphate ; 10-(halomethyl)-10-dodecanol-1-yl-diphosphate ; 11-(halomethyl)-11-dodecanol-1-yl-diphosphate ; 11-(halomethyl)-11-tridecanol-1-yl-diphosphate ; 12-(halomethyl)-12-tridecanol-1-yl-diphosphate ; 12-(halomethyl)-12-tetradecanol-1-yl-diphosphate ; 13-(halomethyl)-13-tetradecanol-1-yl-diphosphate ; 13-(halomethyl)-13-pentadecanol-1-yl-diphosphate ; 14-(halomethyl)-14-pentadecanol-1-yl-diphosphate ; 14-(halomethyl)-14-hexadecanol-1-yl-diphosphate ; 15-(halomethyl)-15-hexadecanol-1-yl-diphosphate ; 15-(halomethyl)-15-heptadecanol-1-yl-diphosphate ; 16-(halomethyl)-16-heptadecanol-1-yl-diphosphate ; 16-(halomethyl)-16-octadecanol-1-yl-diphosphate ; 17-(halomethyl)-17-octadecanol-1-yl-diphosphate ; 17-(halomethyl)-17-nonadecanol-1-yl-diphosphate ; 18-(halomethyl)-18-nonadecanol-1-yl-diphosphate ; 18-(halomethyl)-18-eicosanol-1-yl-diphosphate ; 19-(halomethyl)-19-eicosanol-1-yl-diphosphate ; 19-(halomethyl)-19-heneicosanol-1-yl-diphosphate ; 20-(halomethyl)-20-heneicosanol-1-yl-diphosphate ; 20-(halomethyl)-20-docosanol-1-yl-diphosphate ; 21-(halomethyl)-21-docosanol-1-yl-diphosphate ; and 21-(halomethyl)-21-tricosanol-1-yl-diphosphate.

More particularly, the γδT cell activator can be selected from the group consisting of : 3-(bromomethyl)-3-butanol-1-yl-diphosphate (BrHPP) ; 5-bromo-4-hydroxy-4-methylpentyl pyrophosphonate (CBrHPP) ; 3-(iodomethyl)-3-butanol-1-yl-diphosphate (IHPP) ; 3-(chloromethyl)-3-butanol-1-yl-diphosphate (ClHPP) ; 3-(bromomethyl)-3-butanol-1-yl-triphosphate (BrHPPP) ; 3-(iodomethyl)-3-butanol-1-yl-triphosphate (IHPPP) ; α,γ-di-[3-(bromomethyl)-3-butanol-1-yl]-triphosphate (diBrHTP) ; and α,γ-di-[3-(iodomethyl)-3-butanol-1-yl]-triphosphate (diIHTP).

In an other particular embodiment, the γδT cell activator can be selected from the group consisting of : 3,4-epoxy-3-methyl-1-butyl-diphosphate (Epox-PP) ; 3,4,-epoxy-3-methyl-1-butyl-triphosphate (Epox-PPP) ; α,γ-di-3,4,-epoxy-3-methyl-1-butyl-triphosphate (di-Epox-TP) ; 3,4-epoxy-3-ethyl-1-butyl-diphosphate ; 4,5-epoxy-4-methyl-1-pentyl-diphosphate ; 4,5-epoxy-4-ethyl-1-pentyl-diphosphate ; 5,6-epoxy-5-methyl-1-hexyl-diphosphate ; 5,6-epoxy-5-

ethyl-1-hexyl-diphosphate ; 6,7-epoxy-6-methyl-1-heptyl-diphosphate ; 6,7-epoxy-6-ethyl-1-heptyl-diphosphate ; 7,8-epoxy-7-methyl-1-octyl-diphosphate ; 7,8-epoxy-7-ethyl-1-octyl-diphosphate ; 8,9-epoxy-8-methyl-1-nonyl-diphosphate ; 8,9-epoxy-8-ethyl-1-nonyl-diphosphate ; 9,10-epoxy-9-methyl-1-decyl-diphosphate ; 9,10-epoxy-9-ethyl-1-decyl-diphosphate ; 10,11-epoxy-10-methyl-1-undecyl-diphosphate ; 10,11-epoxy-10-ethyl-1-undecyl-diphosphate ; 11,12-epoxy-11-methyl-1-dodecyl-diphosphate ; 11,12-epoxy-11-ethyl-1-dodecyl-diphosphate ; 12,13-epoxy-12-methyl-1-tridecyl-diphosphate ; 12,13-epoxy-12-ethyl-1-tridecyl-diphosphate ; 13,14-epoxy-13-methyl-1-tetradecyl-diphosphate ; 13,14-epoxy-13-ethyl-1-tetradecyl-diphosphate ; 14,15-epoxy-14-methyl-1-pentadecyl-diphosphate ; 14,15-epoxy-14-ethyl-1-pentadecyl-diphosphate ; 15,16-epoxy-15-methyl-1-hexadecyl-diphosphate ; 15,16-epoxy-15-ethyl-1-hexadecyl-diphosphate ; 16,17-epoxy-16-methyl-1-heptadecyl-diphosphate ; 16,17-epoxy-16-ethyl-1-heptadecyl-diphosphate ; 17,18-epoxy-17-methyl-1-octadecyl-diphosphate ; 17,18-epoxy-17-ethyl-1-octadecyl-diphosphate ; 18,19-epoxy-18-methyl-1-nonadecyl-diphosphate ; 18,19-epoxy-18-ethyl-1-nonadecyl-diphosphate ; 19,20-epoxy-19-methyl-1-eicosyl-diphosphate ; 19,20-epoxy-19-ethyl-1-eicosyl-diphosphate ; 20,21-epoxy-20-methyl-1-heneicosyl-diphosphate ; 20,21-epoxy-20-ethyl-1-heneicosyl-diphosphate ; 21,22-epoxy-21-methyl-1-docosyl-diphosphate ; and 21,22-epoxy-21-ethyl-1-docosyl-diphosphate.

In a further particular embodiment, the γδT cell activator can be selected from the group consisting of : 3,4-epoxy-3-methyl-1-butyl-diphosphate (Epox-PP) ; 3,4,-epoxy-3-methyl-1-butyl-triphosphate (Epox-PPP) ; α,γ-di-3,4,-epoxy-3-methyl-1-butyl-triphosphate (di-Epox-TP) ; and uridine 5'-triphosphate -(3,4-époxy methyl butyl) (Epox-UTP).

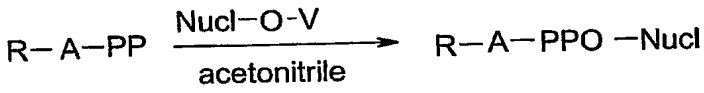
In a preferred embodiment, the γδT cell activator can be selected from the group consisting of : (E)-4-hydroxy-3-methyl-2-butenyl pyrophosphate (HDMAPP) and (E)-5-hydroxy-4-methylpent-3-enyl pyrophosphonate (CHDMAPP).

These compounds may be produced according to various techniques known per se in the art, some of which being disclosed in PCT Publications nos. WO 00/12516, WO 00/12519, WO 03/050128, and WO 03/009855, the disclosures of which are incorporated herein by reference.

In a most preferred embodiment, the γδT cell activator is selected from the group consisting of HDMAPP, CHDMAPP, Epox-PP, BrHPP and CBrHPP, more preferably HDMAPP, CHDMAPP, BrHPP and CBrHPP, still more preferably HDMAPP.

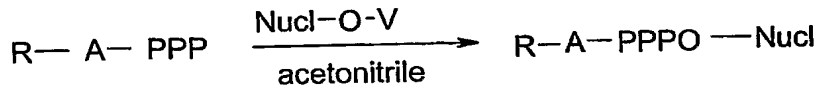
Alternatively, although potentially less efficient, other activators for use in the present invention are phosphoantigens disclosed in WO 95/20673, isopentenyl pyrophosphate (IPP) (US 5,639,653) and 3-methylbut-3-enyl pyrophosphonate (C-IPP). The disclosures of both references are incorporated herein by reference.

Compounds comprising a nucleoside as Y group can be prepared, for example, by the following reactions. Depending on the type and reactivity of the functional groups provided by Y, the professional is able to adapt the following examples, if necessary including the phases of protection/non-protection of the sensitive functional groups or those that can interact with the coupling reaction.



Reaction A

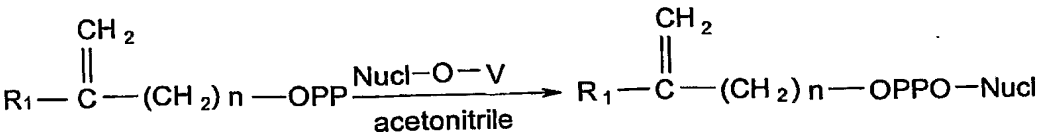
or

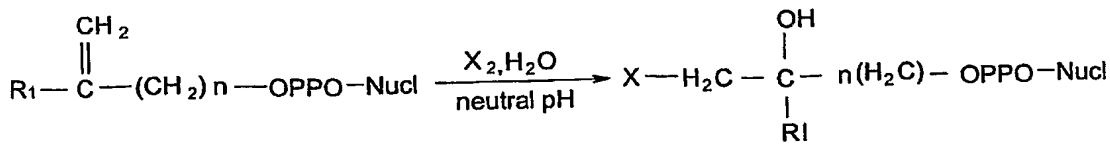


Reaction B

where –O-V is a good group beginning with V chosen, for example, from among tosyle, mesyle, triflyle, brosyle or bromium, PP represents the pyrophosphate group, PPP represents the triphosphate group, R-A- has the above mentionned meaning and Nucl is a nucleoside. Preferably, Nucl-O-V is selected from the group consisting of : 5'-O-Tosyladenosine, 5'-O-Tosyluridine, 5'-O-Tosylcytidine, 5'-O-Tosylthymidine or 5'-O-Tosyl-2'-deoxyadenosine.

For example, for the compound with R of group 1), the reaction procedure can be the following:





where –O-V is a good group beginning with V chosen, for example, from among tosyle, mesyle, triflyle, brosyle or bromium, PP represents the pyrophosphate group and Nucl is a nucleoside. Preferably, Nucl-O-V is selected from the group consisting of : 5'-O-Tosyladenosine, 5'-O-Tosyluridine, 5'-O-Tosylcytidine, 5'-O-Tosylthymidine or 5'-O-Tosyl-

2'-deoxyadenosine as described in Davisson et al, (1987), the disclosure of which is incorporated herein by reference.

Neutral pH is a nucleophile substitution reaction that can be carried out in conditions similar to those described by Davisson et al, (1987); and Davisson et al. (1986), the disclosures of which are incorporated herein by reference.

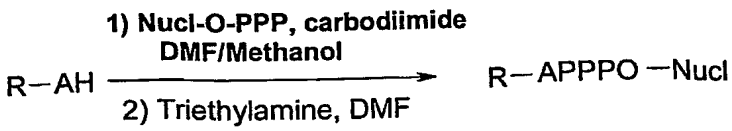
This reaction can also be used to prepare compound comprising a monosaccharide as group Y. In this case, Nucl-O-V is replaced by MonoSac-O-V, wherein Monosac is monosaccharide. For example, it is possible to use the MonoSac-O-Y group corresponding to compound Methyl-6-O-tosyl-alpha-D-galactopyranoside as described in publication Nilsson and Mosbach, (1980) , incorporated herein by reference, or the commercially available mannose triflate compound.

This reaction can further be used to prepare compound comprising a oligosaccharide as group Y. In this case, Nucl-O-V is replaced by oligoSac-O-V, wherein oligoSac is an oligosaccharide. For example, it is possible to use the oligoSac-O-Y group corresponding to compound 6^{A}-O-p-Toluenesulfonyl-ß-cyclodextrin as described in publication (Organic syntheses, Vol. 77, p 225-228, the disclosure of which is incorporated herein by reference).

This reaction can be used to prepare compound comprising a polysaccharide as group Y. In this case, Nucl-O-V is replaced by polySac-O-V, wherein polySac is a polysaccharide. For example, it is possible to use the polySac-O-Y group corresponding to tosylated polysaccharide as described in publication Nilsson et al., (1981); and Nilsson and Mosbach, (1980), the disclosures of which are incorporated herein by reference. This coupling technique based on the activation of the hydroxyl groups of a polysaccharide support by tosylation allows for covalent coupling in an aqueous or an organic medium.

This reaction can also be used for preparing compound comprising an aldehyde derivative as group Y by choosing, instead of Nucl, a derivative including a protected aldehyde function in the form of an acetal or any other group protecting this function.

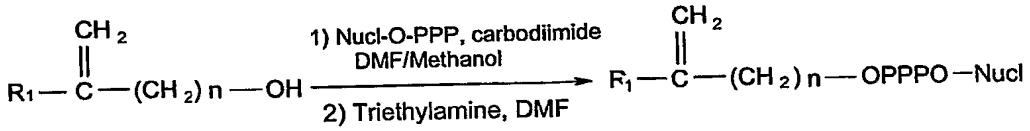
Alternatively, compounds comprising a nucleoside as Y group can be prepared by the following reaction:

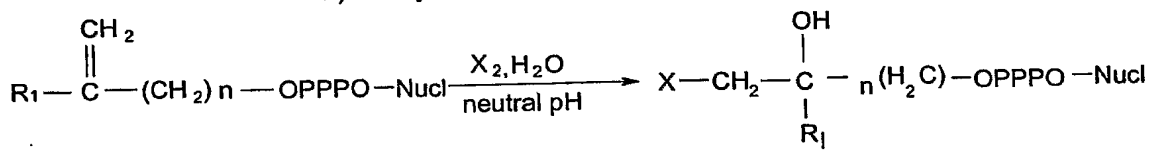


Reaction C

where PPP represents the triphosphate group, R-A has the above mentionned meaning, DMF is dimethylformamide, and Nucl is a nucleoside. This reaction can be carried out in conditions similar to those described by Knorre et al.(1976), or by Bloom et al., United States Patent No. 5,639,653 (1997), the disclosures of which are incorporated herein by reference, from alcohol and a nucleotide with formula Nucl-O-PPP.

For example, for the compound with R of group 1), the reaction procedure can be the following:



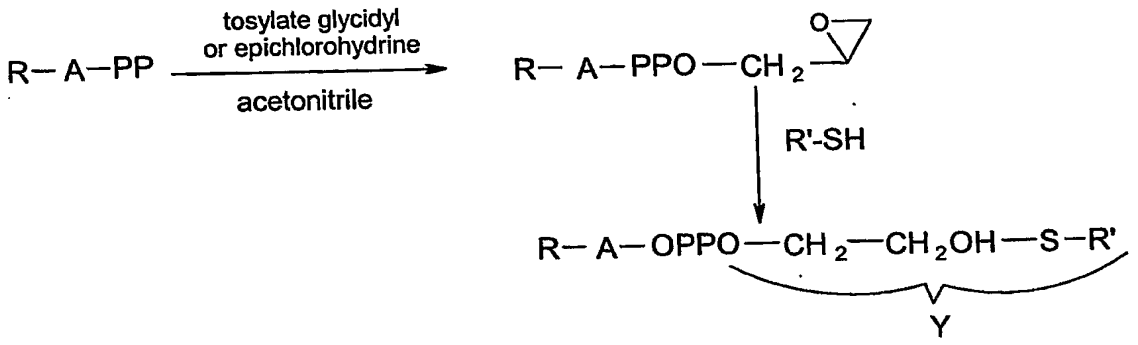


where PPP represents the triphosphate group, DMF is dimethylformamide, and Nucl is a nucleoside.

This reaction can also be applied to the preparation of oligonucleotides 5'-triphosphate γ-esters as indicated by the authors of publication Knorre et al. (1976).

Compounds comprising a nucleic acid as Y group, more particularly a ribonucleic acid, can be prepared in conditions similar to those described in publication F. Huang et al (1997). The authors describe a universal method from catalytic RNA that is applicable to any molecule comprising a free terminal phosphate group. Compounds structurally related to the phosphohalohydrine group such as isopentenyl pyrophosphate or thiamine pyrophosphate are used or mentioned by these authors (see p. 8968 of F. Huang et al (1997)). It should also be noted that the experimental conditions for the coupling procedure (in particular pH conditions) described in the section « Reaction of Isolate 6 pppRNA with phosphate containing Nucleophiles » on page 8965 are compatible with the presence of a halohydrine function.

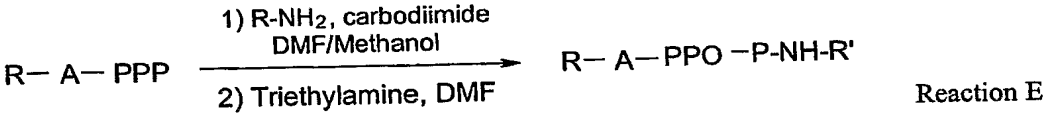
Compounds comprising an amino acid, a peptide or a protein derivative as Y group can be obtained using the well known reactivity of their primary amine or thiol function on an epoxyde function (S_N2 reaction). This type of coupling classically involves an intermediate group still called "linker" bearing an epoxyde function. An example of a reaction procedure using this type of coupling is provided below.



Reaction D

where PP represents the pyrophosphate group, R-A has the above mentionned meaning and R'-SH is an amino acid, a peptide or a protein derivative. The first phase can be carried out in conditions similar to those described by Davisson et al. (1987) and Davisson et al, (1986), the disclosures of which are incorporated herein by reference, from the tetrabutylammonium salt of the initial compound and commercially available compounds such as glycidyl tosylate or epichlorohydrine. This reaction can also be carried out with thriphosphate compounds. Alternatively, a primary amine R'-NH_2 can be used instead of R'-SH. Without the reaction with R'-SH, the first reaction can be used to prepare compound comprising an epoxyde derivative.

Alternatively, compounds comprising an amino acid, a peptide or a protein derivative as Y group can be prepared by the following reaction:



Reaction E

where PPP represents the triphosphate group, PP represents the pyrophosphate group, P represents the phosphate group, R-A has the above mentionned meaning and R'-NH is an amino acid, a peptide or a protein derivative. The reaction can be carried out in conditions similar to those described by Knorre et al. (1976), the disclosure of which is incorporated herein by reference, from compound (R-A-PPP) and an amino acid, peptide or a protein with formula R-NH_2. This reaction involves the protection of the sensitive functions of compound R-NH_2 or can react with the carbodiimide (in particular, the carboxyl function).

Tri or tetra-n-butylammonium salts of phosphoric, pyrophosphoric, triphosphoric, tetra-phosphoric or polyphosphoric acid can be prepared from commercially available corresponding acids. Derivatives with a related structure such as derivatives of methanetrisphosphonic acid described in publication Liu et al (1999), the disclosure of which is incorporated herein by

reference, can also be prepared according to the reaction procedure.The above mentioned reactions can be extrapolated to a very large spectrum of molecules or biomolecules by using the reactivity of the hydroxyl, amine, phosphate or thiol functions. Thereby, inositol derivatives can be prepared according to reactions A or B by activation of the hydroxyl function. Derivatives of folic acid (vitamin B9) or tetrahydrofolic acid can be prepared according to reactions D or E by calling on the reactivity of the primary amine function.

Of course, other types of coupling can be considered and the professional can have access to a large choice of reactions.

Thereby, coupling by phosphorylation of carboxylic acid or phenol groups can be used for the formation of fatty acid, lipid or certain flavonoid derivatives.

Each of the foregoing references relating to compounds and their synthesis are incorporated herein by reference.

As discussed, preferred compounds are selected which increase the biological activity of γδ T cells, preferably increasing the activation of γδ T cells, particularly increasing cytokine secretion from γδ T cells or increasing the cytolytic activity of γδ T cells, with or without also stimulating the expansion of γδ T cells. For example, a γδ T cell activator allows the cytokine secretion by γδ T cells to be increased at least 2, 3, 4, 10, 50, 100-fold, as determined in vitro. Cytokine secretion and cytolytic activity can be assessed using any appropriate in vitro assay, or those described herein.

In another aspect, the present invention relates to methods for the treatment of a carcinoma or viral infection, preferably a urinary or bladder cancer or an HPV infection, where the γδ T cell activator is administered in an amount and under conditions sufficient to stimulate the expansion of the γδ T cell population in a subject, particularly to reach 30-90% of total circulating lymphocytes, typically 40-90%, more preferably from 50-90%. In typical embodiments, the invention allows the selective expansion of γδ T cells in a subject, to reach at least 20%, 30% or 40% of total circulating lymphocytes. Percentage of total circulating lymphocytes can be determined according to methods known in the art. A preferred method for determining the percentage of γδ T cells in total circulating lymphocytes is by flow cytometry.

In another aspect, the present invention relates to methods for the treatment of a carcinoma or viral infection, preferably a urinary or bladder cancer or an HPV infection, where the γδ T cell activator is administered in an amount and under conditions sufficient to stimulate the expansion of the γδ T cell population in a subject, particularly to increase by more than 2-fold the number of γδ T cells in a subject, typically at least 10-fold, more preferably at least 20-fold. In another aspect, the present invention relates to methods for the treatment of a bladder cancer, where the γδ T cell activator, especially a γδ T cell activator according to formulas I to XVII, is administered in an amount and under conditions sufficient to stimulate the expansion of the γδ T cell population in a subject, particularly to reach a circulating γδ T cell count of at least 500 γδ T cells/mm3 in a subject, typically at least 1000 γδ T cells/mm3, more preferably at least 2000 γδ T cells/mm3. The number of γδ T cells and circulating γδ T cell count in a subject is preferably assessed by obtaining a blood sample from a patient before and after administration of said γδ T cell activator and determining the difference in number of γδ T cells present in the sample.

Preferably, dosage (single administration) of a γδ T cell activator compound of formula I for treatment is between about 1 μg/kg and about 1.2 g/kg. It will be appreciated that the above dosages related to a group of compounds, and that each particular compound may vary in optimal doses, as further described herein for exemplary compounds. Nevertheless, compounds are preferably administered in a dose sufficient to significantly increase the biological activity of γδ T cells or to significantly increase the γδ T cell population in a subject. Said dose is preferably administered to the human by intravenous (i.v.) administration during 2 to 180 min, preferably 2 to 120 min, more preferably during about 5 to about 60 min, or most preferably during about 30 min or during about 60 min. In preferred exemplary compounds, a compound of formula II to XI is administered in a dosage (single administration) between about 0.1 mg/kg and about 1.2 g/kg, preferably between about 10 mg/kg and about 1.2 g/kg, more preferably between about 5 mg/kg and about 100 mg/kg, even more preferably between about 5 μg/kg and 60 mg/kg. Most preferably, dosage (single administration) for three-weekly or four-weekly treatment (treatment every three weeks or every third week) is between about 0.1 mg/kg and about 1.2 g/kg, preferably between about 10 mg/kg and about 1.2 g/kg, more preferably between about 5 mg/kg and about 100 mg/kg, even more preferably between about 5 μg/kg and 60 mg/kg. In preferred exemplary compounds, a compound of formula XII to XVII, is administered in a dosage (single administration) between about 1 μg/kg and about 100 mg/kg, preferably between about 10 μg/kg and about 20 mg/kg, more preferably between about 20 μg/kg and about 5 mg/kg, even more preferably between about 20 μg/kg and 2.5 mg/kg. Most preferably, dosage (single administration) for three-weekly or four-weekly treatment (treatment every three

weeks or every third week) is between about 1 μg/kg and about 100 mg/kg, preferably between about 10 μg/kg and about 20 mg/kg, more preferably between about 20 μg/kg and about 5 mg/kg, even more preferably between about 20 μg/kg and 2.5 mg/kg. Further detail on dosages and administration and examples of dose response experiments using γδ T cell activator in mice and primate models are provided in co-pending PCT Application no. PCT/FR03/03560 filed 2 December 2003, the disclosure of which is incorporated herein by reference.

Treatment regimens

(a) *Bladder Cancer*

Treatment with a mycobacterial antigen and a γδ T cell activator can be carried out according to any suitable administration regimen. The γδ T cell activator may be administered through any of several different routes, typically by injection or oral administration. Injection may be carried out into various tissues, such as by intravenous, intra-peritoneal, intra-arterial, intra-muscular, intra-dermic, subcutaneous, etc. Particularly preferred is intravenous injection. The γδ T cell activator can be administered before, at the same time or after the mycobacterial antigen is administered. Generally, the γδ T cell activator will be administered no more than several (4, 5, 6, or 7) days before or after treatment with the mycobacterial antigen. Most preferably, however, the γδ T cell activator is administered at substantially the same time as the mycobacterial antigen is administered, preferably within 48 hours, 24 hours or more preferably within 12 or within 6 hours of treatment with the the mycobacterial antigen. For example, in the regimen of the Examples, the γδ T cell activator is administered several hours before administration of the mycobacterial antigen.

In one aspect, the γδ T cell activator is administered once during the course of mycobacterial antigen therapy. More preferably, however, the γδ T cell activator is administered several times. Most preferably, the γδ T cell activator is administered according to a regimen in which γδ T cell activity, preferably the γδ T cell rate (number of γδ T cells), is allowed to return to substantially basal rate prior to a second administration of the compound. As provided in co-pending PCT Application no. PCT/FR03/03560 filed 2 December 2003, the disclosure of which is incorporated herein by reference, at least about one week, but more preferably at least about two weeks, are required for a patient's γδ T cell rate to return to substantially basal rate.

The course of a preferred cycle for administering the γδ T cell activator is an at least 1-weekly cycle, but more preferably at least a 2-weekly cycle (at least about 14 days), or more preferably at least 3-weekly or 4-weekly, though cycles anywhere between 2-weekly and 4-weekly are preferred. Also effective and contemplated are cycles of up to 8-weekly, for example 5-weekly, 6-weekly, 7-weekly or 8-weekly. In a preferred embodiment, the γδ T cell activator is administered only the first day of a 2-weekly to 4-weekly, or preferably 3 weekly, cycle.

In an exemplary embodiment, the mycobacterial antigen is administered on a 1-weekly cycle for 6 weeks, and the γδ T cell activator occurs on the first day of a 2-weekly to 4-weekly cycle (that is, an about 14 to 28 day weeks repeating cycle). In a preferred embodiment, the mycobacterial antigen is administered on a 1-weekly cycle for 6 weeks and the γδ T cell activator is administered only the first day of the 2-weekly to 4-weekly, or preferably 3 weekly, cycle.. Preferably the γδ T cell activator is administered for at least substantially the duration of mycobacterial antigen treatment. For example, a 3-weekly cycle is used for the γδ T cell activator and a 1-weekly cycle is used for the mycobacterial antigen, both over a course of six weeks according to the following scheme:

Day 0: mycobacterial antigen and γδ T cell activator
Day 7: mycobacterial antigen
Day 14: mycobacterial antigen
Day 21: mycobacterial antigen and γδ T cell activator
Day 28: mycobacterial antigen
Day 35: mycobacterial antigen
Day 42: (optional): γδ T cell activator

In other exemplary administration regimens, particularly in maintenance therapy, a 3-weekly cycle is used for both the γδ T cell activator and the mycobacterial antigen. Preferably the γδ T cell activator and the mycobacterial antigen are administered on the same day.

As mentioned, a subject will preferably be treated for at least two cycles of γδ T cell activator, or more preferably for at least three cycles, or for at least one cycle of mycobacterial antigen therapy, preferably at least one cycle of 1-weekly mycobacterial antigen administration for a 6 week treatment cycle. In other aspect, treatment may continue for a greater number of cycles, for example at least 4, 5, 6 or more cycles can be envisioned. At the end of each cycle, the cycle of dosing may be repeated for as long as clinically tolerated and the tumor is under control or until tumor regression. In exemplary mycobacterial antigen regimens, administrations of

mycobacterial antigen take place 1-weekly for 6 weeks, followed by an interval (for example 6 weeks), followed by administrations of mycobacterial antigen 3-weekly for a desired duration, such as at least 6 months or 12 months for maintenance therapy.

In other embodiments, the methods of the invention comprises further administering a cytokine. While the compounds of the invention may be used with or without further administration, in a preferred aspect a cytokine can be administered, wherein said cytokine is capable of increasing the expansion of a γδ T cell population treated with a γδ T cell activator compound, preferably wherein the cytokine is capable of inducing an expansion of a γδ T cell population which is greater than the expansion resulting from administration of the γδ T cell activator compound in the absence of said cytokine. A preferred cytokine is an interleukin-2 polypeptide.

A cytokine having γδ T cell proliferation inducing activity, most preferably the interleukin-2 polypeptide, is administered at low doses, typically over a period of time comprised between 1 and 10 days. The γδ T cell activator is preferably administered in a single dose, and typically at the beginning of a cycle.

In preferred aspects, a cytokine, most preferably IL-2, is administered daily for up to about 10 days, preferably for a period of between about 3 and 10 days, or most preferably for about 7 days. Preferably, the administration of the cytokine begins on the same day (e.g. within 24 hours of) as administration of the γδ T cell activator. It will be appreciated that the cytokine can be administered in any suitable scheme within said regimen of between about 3 and 10 days. For example, in one aspect the cytokine is administered each day, while in other aspects the cytokine need not be administered on each day.

(b) HPV infection

Infections caused by human papilloma virus (HPV) using the Mycobacterium that can be treated according to the methods of the invention may include cutaneous and genital warts in humans, including verruca vulgaris and condyloma acuminatum, cervical intraepithelial neoplasia and genital carcinomas. In general, the treatment is applicable to any disease condition caused by HPV in humans including penile, intraurethral, perianal, intra-anal or perineal infections in men and cervical, vaginal, perigenital, intra-urethral, intra-anal and perineal infections in women, including condylomata acuminata, penile cancer, Bowen's disease, cervical cancer, head and neck cancer, laryngeal papillomatosis and laryngeal carcinoma.

The mycobacterial antigen treatment may be effected by application of the Mycobacterium antigen in a suitable carrier to the region of infection, which may involve topical application to cutaneous, penile and perianal areas, or intraurethral application to the urogenital tract. The treatment may involve a single or a plurality of doses applied at time intervals. The individual dosage level may be about 1 mg to about 500 mg attenuated BCG while the time interval between doses may vary from about 1 to about 30 days. The number of treatments applied is from 1 to about 30 treatments. The mycobacterial antigen and γδ T cell activator combination treatment is can be used alone or may be preceded by laser or other surgical or topical therapy.

The γδ T cell activator may be administered as for the treatment of bladder carcinoma, such as through any of several different routes, typically by injection or oral administration. Injection may be carried out into various tissues, such as by intravenous, intra-peritoneal, intra-arterial, intra-muscular, intra-dermic, subcutaneous, etc. Particularly preferred is intravenous injection. The γδ T cell activator can be administered before, at the same time or after the mycobacterial antigen is administered. Generally, the γδ T cell activator will be administered no more than several (4, 5, 6, or 7) days before or after treatment with the mycobacterial antigen. Most preferably, however, the γδ T cell activator is administered at substantially the same time as the mycobacterial antigen is administered, preferably within 48 hours, 24 hours or more preferably 12 or 6 hours of treatment with the the mycobacterial antigen. For example, in the regimen of the Examples, the γδ T cell activator is administered several hours before administration of the mycobacterial antigen.

In one aspect, the γδ T cell activator is administered once during the course of mycobacterial antigen therapy. More preferably, however, the γδ T cell activator is administered several times. Most preferably, the γδ T cell activator is administered according to a regimen in which γδ T cell activity, preferably the γδ T cell rate (number of γδ T cells), is allowed to return to substantially basal rate prior to a second administration of the compound. The course of a preferred cycle for administering the γδ T cell activator is an at least 1-weekly cycle, but more preferably at least a 2-weekly cycle (at least about 14 days), or more preferably at least 3-weekly or 4-weekly, though cycles anywhere between 2-weekly and 4-weekly are preferred. Also effective and contemplated are cycles of up to 8-weekly, for example 5-weekly, 6-weekly, 7-weekly or 8-weekly. In a preferred embodiment, the γδ T cell activator is administered only the first day of a 2-weekly to 4-weekly, or preferably 3 weekly, cycle.

In an exemplary embodiment, the mycobacterial antigen is administered on a 1-weekly cycle for about 6 weeks, or for at least 4, 6, 8, 10 or 12 weeks, and the γδ T cell activator occurs on the first day of a 2-weekly to 4-weekly cycle (that is, an about 14 to 28 day weeks repeating cycle). In a preferred embodiment, the mycobacterial antigen is administered on a 1-weekly cycle for 6 weeks and the γδ T cell activator is administered only the first day of the 2-weekly to 4-weekly, or preferably 3 weekly, cycle. Preferably the γδ T cell activator is administered for at least substantially the duration of mycobacterial antigen treatment. For example, a 3-weekly cycle is used for the γδ T cell activator and a 1-weekly cycle is used for the mycobacterial antigen, both over a course of six weeks according to the following scheme:

Day 0: mycobacterial antigen and γδ T cell activator
Day 7: mycobacterial antigen
Day 14: mycobacterial antigen
Day 21: mycobacterial antigen and γδ T cell activator
Day 28: mycobacterial antigen
Day 35: mycobacterial antigen
Day 42: (optional): γδ T cell activator

In other exemplary administration regimens, a 3-weekly cycle is used for both the γδ T cell activator and the mycobacterial antigen. Preferably the γδ T cell activator and the mycobacterial antigen are administered on the same day. In other embodiments, a cytokine may additionally be administered, according to a regimen as described for the treatment of bladder carcinoma.

For topical use in the treatment of HPV infection, the Mycobacterium may be formulated with a keratolytic agent for topical application to the region of infection, particularly as a cream for adherent application to the region of infection. The keratolytic agent may be salicylic acid, which may be powdered. The keratolytic agent may be present in an amount of about 0.1 to about 50 wt%, preferably about 1 to about 10 wt%. The composition which is applied to the area of infection may take any desired form, for example, a cream, a powder or ointment. Any desired form of application may be employed, including slow-release systems, plasters and transdermal systems.

The following examples will serve to further illustrate the present invention without, at the same time, however, constituting any limitation thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof

which, after reading the description herein, may suggest themselves to those skilled in the artwithout departing from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLES

EXAMPLE 1

Synthesis of BrHPP

All glassware and equipment were dried for several hours prior to use. Unless otherwise stated, the reagents and starting material were from Fluka. Trisodium (*R,S*)-3-(bromomethyl)-3-butanol-1-yl-diphosphate (BrHPP) was produced as white amorphous powder by the following procedure. Tosyl chloride (4.8 g, 25 mmol) and 4-(*N,N*-dimethylamino-) pyridine (3.4 g, 27.5 mmol; Aldrich) were mixed under magnetic stirring with 90 ml of anhydrous dichloromethane in a 250-ml three-necked flask cooled in an ice bath. A solution of 3-methyl-3-butene-1-ol (2.2 g, 25 mmol) in about 10 ml of anhydrous dichloromethane was then slowly introduced with a syringe through a septum in the flask, and the ice bath was then removed. The reaction was monitored by silica gel TLC (pentane/ethyl acetate, 85:15 (v/v)). After 2 h with constant stirring, the mixture was precipitated by dilution into 1 liter of hexane and filtered, and the filtrate was concentrated under reduced pressure. This filtration/suspension step was repeated using diethyl ether, and the resulting oil was purified by liquid chromatography on silica gel (pentane/ethyl acetate, 85:15 (v/v)), yielding a yellow oil of 3-methyl-3-butene-1-yl-tosylate (5.6 g, 23.5 mmol, 94% yield) kept under dry N_2 at 4 °C (positive mode ESI-MS: *m/z* 241 $[M + H]^+$; *m/z* 258 $[M + NH_4]^+$; *m/z* 263 $[M + Na]^+$; MS^2 of *m/z* 258: *m/z* 190 (C_5H_8 loss)).

Disodium dihydrogen pyrophosphate (51.5 mmol, 11.1 g) dissolved in 100 ml of deionized water (adjusted to pH 9 with NH_4OH) was passed over a cation exchange DOWEX 50WX8 (42 g, 200 meq of form H^+) column and eluted with 150 ml of deionized water (pH 9). The collected solution was neutralized to pH 7.3 using tetra-*n*-butyl ammonium hydroxide and lyophilized. The resulting hygroscopic powder was solubilized with anhydrous acetonitrile and further dried by repeated evaporation under reduced pressure. The resulting Tris (tetra-*n*-butyl ammonium) hydrogenopyrophosphate (97.5% purity by HPAEC; see below) was stored (concentration, ~0.5 M) at −20 °C in anhydrous conditions under molecular sieves. 100 ml of a solution containing 50 mmol of Tris (tetra-*n*-butyl ammonium) hydrogenopyrophosphate (0.5 M, 2.5 eq) in anhydrous acetonitrile under magnetic stirring in a 250-ml three-necked flask cooled in an ice bath were slowly mixed with 20 mmol (4.8 g) of 3-methyl-3-butene-1-yl-tosylate introduced via a septum with a syringe. After 20 min, the ice bath was withdrawn, and the reaction was left under agitation at room temperature for 24 h. The reaction was analyzed by HPAEC (see below), evaporated, and diluted into 50 ml of a mixture composed of a solution (98 % volume) of ammonium hydrogenocarbonate (25 mM) and 2-propanol (2 volume %). The

resulting mixture was passed over a cation exchange DOWEX 50WX8 (NH_4^+, 750 meq) column formerly equilibrated with 200 ml of the solution (98 % volume) of ammonium hydrogenocarbonate (25 mM) and 2-propanol (2 volume %). The column was eluted with 250 ml of the same solution at a slow flow and collected in a flask kept in an ice bath. The collected liquid was lyophilized, and the resulting white powder was solubilized in 130 ml of ammonium hydrogenocarbonate (0.1 M) and completed by 320 ml of acetonitrile/2-propanol (v/v). After agitation, the white precipitate of inorganic pyro- and mono-phosphates was eliminated by centrifugation (2100 × *g*, 10 °C, 8 min). This procedure was repeated three times, the supernatant was collected and dried, and the resulting oil was diluted in 120 ml of water. Remainders of unreacted tosylates were extracted three times by chloroform/methanol (7:3 (v/v)) in a separatory funnel, and the water phase was finally lyophilized. The resulting white powder was again washed twice by acetonitrile/chloroform/methanol (50:35:15 (v/v)) and dried under gentle N_2 flow. 11.25 mmol of pure 3-methyl-3-butene-1-yl-pyrophosphate triammonium salt were obtained by this procedure (75% yield) and were then dissolved in 200 ml of water for oxidation. For 6 mmol of 3-methyl-3-butene-1-yl-pyrophosphate, an aqueous solution of Br_2 (0.1 M) kept at 4 °C was added dropwise until appearance of a persistent yellowish color, yielding after evaporation 5.8 mmol (2.3 g) of an acidic solution (pH 2.1) of BrHPP, which was immediately neutralized by passing over DOWEX 50WX8-200 (NH_4^+, 48 meq). The ammonium salt of BrHPP obtained after lyophilization was dissolved in water and separated from bromides by passing through Dionex OnGuard-Ag (2 meq/unit) cartridges and an on-line column of (100 meq, 21 g) DOWEX 50WX8-200 (Na^+) eluted by milli-Q water. Colorless stock solutions of BrHPP (Na^+) were filtered over Acrodisc 25 membranes of 0.2 µM and kept as aliquots at −20 °C.

HPLC-- Final purification of BrHPP was achieved by HPLC (Spectra system P1000 XR device) on an analytic Symmetry 5 µ C18 column (Waters) eluted at 1 ml/min and 20 °C with the ternary gradient indicated below. Upstream of detectors, a split of eluent distributes 190 µl/min in the online MS detector (see below), and the remaining 810 µl/min was sent to the Waters 996 photodiode array detector. Single wavelength detection at $\lambda = 226$ nm was of 7 milliabsorbance units for 6 µg of BrHPP injected in 25 µl (Rheodyne injector). The gradient program was as follows: solvent A, acetonitrile; solvent B, 50 mM ammonium acetate; solvent C, water; 0-7 min, 5% B in C; 7.1-11 min, 100% C; 12-15 min, 100% A; 15-17 min, 100% C.

EXAMPLE 2

Synthesis of HDMAPP

Synthesis was carried out substantially as described in Hecht et al, Tetrahedron Letters (2002) 43: 8929.

(E)-4-Chloro-2-methylbut-2-en-1-ol

TiCl4 (285mg, 1.5mmol, 164.5 µL) is dissolved in 3 mL of dry CH2CL2 under N2. The solution is cooled to –80 to –90C, and a solution of 84 mg of commercially available 2-methyl-2-cinyloxirane (98.2 µL, 1 mmol) in 0.4 mL of CH2CL2 is added in dropes with stirring. After 90 min. the reaction mixture is quenched by adding 5 mL of 1N HCl. After warming to room temperature, the phases are separated and the aqueous layer is extracted four times with 20 mL of diethyl ether. The combined organic phases are dried over MgSO4. Evaporation of the solvent and purification by flash chromatography (pentanes/diethyl ether 1:1 v/v) affords 93 mg of pure product.

(E)-1-Hydroxy-2-methylbut-2-enyl 4-diphosphate from (E)-4-Chloro-2-methylbut-2-en-1-ol

A solution containing 227 mg (0.25 mmol) of tris (tetra-n-butylammonium) hydrogen pyrophosphate in 300 µL of MeCN is added slowly at room temperature to a solution of (E)-4-Chloro-2-methylbut-2-en-1-ol (25 mg, 0.21 mmol) in 250 µL of MeCN affording an orange-red solution. After 2 h, the solvent is removed under reduced pressure. The orange-colored oil is dissolved in 3 mL of H2O, and the solution is passed through a column of DOWEX 50 WX8 (1x4 cm, NH4+ form) that has been equilibrated with 20 mL of 25 mM NH4HCO3. The column is developed with 20 mL of 25 mM NH4HCO3. Fractions are combined and lyophilized to yield 0.19 mmol of pure product (90%).

EXAMPLE 3

Administration of BrHPP for treatment of superficial bladder cancer in humans

ImmuCyst® (Bacillus Calmette-Guérin (BCG), substrain Connaught) is commercialised by Aventis Pasteur SA, France. ImmuCyst® is made from a culture of an attenuated strain of living bovine tubercle bacillus Mycobacterium bovis. Phosphostim (Innate Pharma, Marseille, France) is based on a new chemical entity, the drug substance Bromohydrin Pyrophosphate (BrHPP), which is a specific agonist of immune competent cells namely the Vγ9Vδ2 T cell subpopulation bearing anti-tumor activity. Phosphostim (BrHPP, 200 mg) is the intravenous formulation of BrHPP for cancer immunotherapy.

Treatment begins between 7 to 14 days after biopsy or transurethral resection. On the same day, ImmuCyst® is administered as intravesical treatment of the urinary bladder and Phosphostim is administered intravenously. The induction treatment comprises 6 weekly intravesical treatments with ImmuCyst®. Each treatment dose of ImmuCyst® comprises one 81 mg vial of ImmuCyst®. A patient receives repeated cycles of Phosphostim treatment every 3 weeks. The cycle consists of one administration by infusion of Phosphostim. Phosphostim can be administered in a dose of about 200 mg/m^2 or about 600 mg/m^2, although the Phosphostim dose can be between 200 mg/m^2 (5 mg/kg) (corresponding to 118 mg-equivalent of BrHPP anionic form) and about 1000 mg/ m^2 and will be determined in a dose ranging study. Optionally, each administration of Phosphostim is combined with an administration of 1 million IU/m^2/day of IL-2 (for a total duration of 7 days).

ImmuCyst® is preferably dosed and administered according to the manufacturer's instructions. Each dose (1 reconstituted vial) is further diluted in an additional 50 mL of sterile, preservative-free saline for a total of 53 mL. A urethral catheter is inserted into the bladder under aseptic conditions, the bladder is drained, and then 53 mL suspension of ImmuCyst® is instilled slowly by gravity, following which the catheter is withdrawn. The patient retains the suspension for as long as possible for a total of up to two hours. During the first 15 minutes following instillation, the patient should lie prone. Thereafter, the patient is then allowed to be up. At the end of 2 hours, all patients should void in a seated position for environmental safety reasons. Patients should be instructed to maintain adequate hydration.

Phosphostim (BrHPP, 200 mg) is a freeze-dried apyrogenic sterile white powder to be reconstituted in solution for infusion. Each vial of Phosphostim (BrHPP, 200mg) contains 200 mg of BrHPP anionic form and 50mg the excipient alpha-lactose monohydrate (USP). Phosphostim is for immediate and single use following first opening and reconstitution. Phosphostim is reconstituted immediately prior to use with 2 ml of water for injections to make a 100 mg/ml solution. The needed quantities of reconstituted product are diluted in a total volume 100 ml of ringer lactate buffer infusion vehicle. The diluted solution is clear and colorless. Phosphostim is administered intravenously over 1 hour.

CLAIMS

1- A method for treating a carcinoma or viral infection in a patient, comprising administering to a patient in need thereof an amount of a Mycobacterium antigen and a γδT cell activator effective to treat said carcinoma or viral infection.

2- The method according to claim 1, wherein said Mycobacterium antigen is an attenuated Mycobacterium strain.

3- The method according to claim 2, wherein said attenuated Mycobacterium strain is an attenuated Mycobacterium bovis.

4- The method according to any one of claims 1 to 4, wherein said Mycobacterium antigen is administered locally to a site of disease.

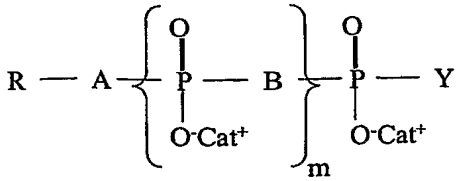
5- The method according to claim 4, wherein said Mycobacterium antigen is administered topically to cutaneous, penile and perianal areas, or intraurethrally application to the urogenital tract.

6- The method according to claim 4, wherein said Mycobacterium antigen is administered intravesicularly into the bladder.

7- The method according to any one of claims 1 to 6, wherein said γδT cell activator is administered systemically.

8- The method according to any one of claims 1 to 6, wherein said γδT cell activator is administered within 48 hours of administration of said Mycobacterium antigen.

9- The method according to claim 1, wherein said γδT cell activator is a compound of formula (I):



Formula (I)

wherein Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including proton);

m is an integer from 1 to 3;

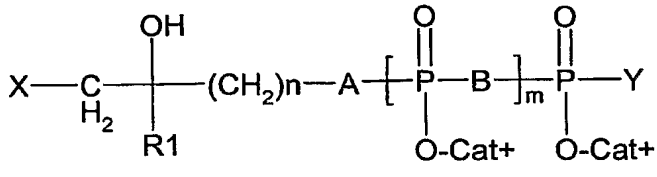
B is O, NH, or any group capable to be hydrolyzed;

Y = O⁻Cat+, a C_1-C_3 alkyl group, a group -A-R, or a radical selected from the group consisting of a nucleoside, an oligonucleotide, a nucleic acid, an amino acid, a peptide, a protein, a monosaccharide, an oligosaccharide, a polysaccharide, a fatty acid, a simple lipid, a complex lipid, a folic acid, a tetrahydrofolic acid, a phosphoric acid, an inositol, a vitamin, a co-enzyme, a flavonoid, an aldehyde, an epoxyde and a halohydrin;

A is O, NH, CHF, CF_2 or CH_2; and,

R is a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C_1-C_{50} hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkylenyl, or an alkynyl, preferably an alkyl or an alkylene, which can be substituted by one or several substituents selected from the group consisting of : an alkyl, an alkylenyl, an alkynyl, an epoxyalkyl, an aryl, an heterocycle, an alkoxy, an acyl, an alcohol, a carboxylic group (-COOH), an ester, an amine, an amino group ($-NH_2$), an amide ($-CONH_2$), an imine, a nitrile, an hydroxyl (-OH), a aldehyde group (-CHO), an halogen, an halogenoalkyl, a thiol (-SH), a thioalkyl, a sulfone, a sulfoxide, and a combination thereof.

10. The method according to claim 9, where said γδ T cell activator is a compound of formula (II):



(II)

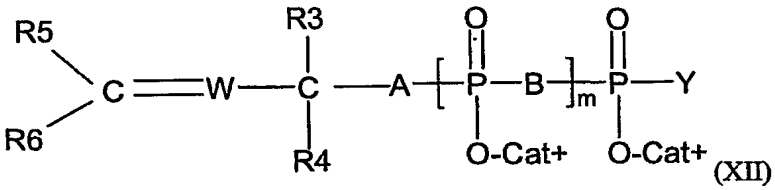
in which X is an halogen (preferably selected from I, Br and Cl), B is O or NH, m is an integer from 1 to 3, R1 is a methyl or ethyl group, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), and n is an integer from 2 to 20, A is O, NH, CHF, CF_2 or CH_2, and Y is O⁻Cat+, or a nucleoside.

11- The method according to claim 10, wherein the compound of formula (II) is BrHPP.

12- The method according to claim 10, wherein the compound of formula (II) is CBrHPP.

13- The method according to claim 10, wherein the compound of formula (II) is epoxPP.

14- The method according to claim 9, where said γδ T cell activator is a compound of formula (XII):



(XII)

in which R_3, R_4, and R_5 , identical or different, are a hydrogen or (C_1-C_3)alkyl group, W is –CH- or –N-, R_6 is an (C_2-C_3)acyl, an aldehyde, an (C_1-C_3)alcohol, or an (C_2-C_3)ester, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), B is O or NH, m is an integer from 1 to 3, A is O, NH, CHF, CF_2 or CH_2, and Y is O⁻Cat+, or a nucleoside.

15- The method according to claim 14, wherein the compound of formula (XII) is HDMAPP.

16- The method according to claim 14, wherein the compound of formula (XII) is CHDMAPP.

17- The method according to any one of claims 1 to 4 or 6 to 16, wherein said Mycobacterium antigen is administered after a transurethal resection.

18- The method according to any one of claims 1 to 4 or 6 to 16, wherein said bladder cancer is a stage 0 bladder cancer.

19- The method according to claim 18, wherein said stage 0 bladder cancer is a non-invasive papillomary carcinoma (TaT1) or a carcinoma in situ (CIS).

20- A pharmaceutical composition comprising a Mycobacterium antigen and a γδT cell activator at an effective dose to treat a carcinoma or viral infection.

21- A kit comprising a pharmaceutical composition comprising a Mycobacterium antigen and a pharmaceutical composition comprising a γδT cell activator, said compositions at effective doses to treat a carcinoma or viral infection when used together in combination therapy.

22- The pharmaceutical composition according to claims 20 or 21, wherein said Mycobacterium antigen is an attenuated Mycobacterium strain.

23- The pharmaceutical composition according to claims 20 or 21, wherein said Mycobacterium antigen and said γδT cell activator are administered simultaneously.

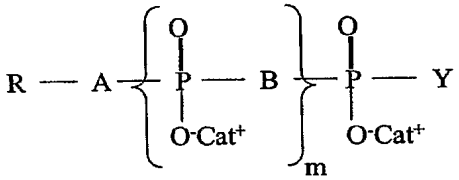
24- The pharmaceutical composition according to claims 20 or 21, wherein said Mycobacterium antigen and said γδT cell activator are administered separately.

25- The pharmaceutical composition according to claim 23 or 24, wherein said Mycobacterium antigen and said γδT cell activator are administered by the same routes.

26- The pharmaceutical composition according to claim 23 or 24, wherein said Mycobacterium antigen and said γδT cell activator are administered by different routes.

27- The pharmaceutical composition according to claim 23 or 24, wherein said Mycobacterium antigen is administered intravesicularly into the bladder.

28- The pharmaceutical composition according to claims 20 or 21, wherein said γδT cell activator is a compound of formula (I) :



Formula (I)

wherein Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including proton);

m is an integer from 1 to 3;

B is O, NH, or any group capable to be hydrolyzed;

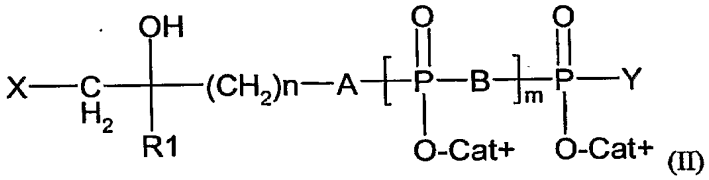
Y = O^-Cat^+, a C_1-C_3 alkyl group, a group -A-R, or a radical selected from the group consisting of a nucleoside, an oligonucleotide, a nucleic acid, an amino acid, a peptide, a protein, a monosaccharide, an oligosaccharide, a polysaccharide, a fatty acid, a simple lipid, a complex lipid, a folic acid, a tetrahydrofolic acid, a phosphoric acid, an inositol, a vitamin, a co-enzyme, a flavonoid, an aldehyde, an epoxyde and a halohydrin;

A is O, NH, CHF, CF_2 or CH_2; and,

R is a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C_1-C_{50} hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group

comprises an alkyl, an alkylenyl, or an alkynyl, preferably an alkyl or an alkylene, which can be substituted by one or several substituents selected from the group consisting of : an alkyl, an alkylenyl, an alkynyl, an epoxyalkyl, an aryl, an heterocycle, an alkoxy, an acyl, an alcohol, a carboxylic group (-COOH), an ester, an amine, an amino group ($-NH_2$), an amide ($-CONH_2$), an imine, a nitrile, an hydroxyl (-OH), a aldehyde group (-CHO), an halogen, an halogenoalkyl, a thiol (-SH), a thioalkyl, a sulfone, a sulfoxide, and a combination thereof.

29. The pharmaceutical composition according to claim 28, where said γδ T cell activator is a compound of formula (II):

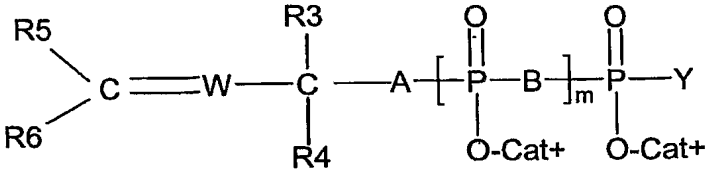


(II)

in which X is an halogen (preferably selected from I, Br and Cl), B is O or NH, m is an integer from 1 to 3, R1 is a methyl or ethyl group, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), and n is an integer from 2 to 20, A is O, NH, CHF, CF_2 or CH_2, and Y is O^-Cat+, or a nucleoside.

30- The pharmaceutical composition according to claim 29, wherein the compound of formula (II) is selected from the group consisting of BrHPP, CBrHPP and epoxPP.

31- The pharmaceutical composition according to claim 28, where said γδ T cell activator is a compound of formula (XII):



(XII)

in which R_3, R_4, and R_5 , identical or different, are a hydrogen or (C_1-C_3)alkyl group, W is –CH- or –N-, R_6 is an (C_2-C_3)acyl, an aldehyde, an (C_1-C_3)alcohol, or an (C_2-C_3)ester, Cat+ represents one (or several, identical or different) organic or mineral cation(s) (including the proton), B is O or NH, m is an integer from 1 to 3, A is O, NH, CHF, CF_2 or CH_2, and Y is O^-Cat+, or a nucleoside.

32- The pharmaceutical composition according to claim 31, wherein the compound of formula (XII) is HDMAPP or CHDMAPP.

ABSTRACT

The present invention relates to compositions and methods useful for treating cancer or viral infection in a mammal, particularly bladder cancer and HPV infection, comprising administering an attenuated strain of Mycobacterium bovis (Bacillus Calmette-Guérin (BCG)), and a γδT cell activator, such that the composition(s) are effective for treating the disease. Methods of making the composition(s) and methods of using them also are disclosed.